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Physical property of food	Automation of food processes
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Editorial

Dear colleagues, authors and readers!

With this issue of the Ukrainian Journal of Food Science, we are confidently entering the 10th year of the publication of the Journal. Although the Journal under the present title has been published since 2013, the scientific tradition of the publisher, the National University of Food Technologies, comes from the middle of the 19th century, and the first collection of scientific papers in the field of food science was published in 1938 under the edition of professor Glib Znamenskyi.

It is pleasant to note that both the scientific and methodological levels of publications submitted to the journal are constantly improving. The geography of authors is also increasing, which indicates the growing popularity of our journal on an international-caliber.

The successes achieved require the editorial board to be more responsible and adhere to the principles of academic integrity, high ethical and professional standards. Taking into account the experience of leading scientific journals, we decide to apply the world-wide principles of transparency in editorial policy and scientific publishing.

In order to improve the quality of our scientific publications, we recommend that you pay attention to the following:

- The introduction should contain a critical analysis of world experience in the direction of research
- Strict adherence to science-related ethical norms avoiding plagiarism, self-plagiarism, as well as a significant level of self-citation should be done.

And a few wishes for the design of articles:

- When preparing an article, follow the instructions for authors.
- Carefully check the correctness of the list of references.
- Write in the third person e.g. ‘this article discusses‘, rather than ‘I discuss’; ‘it is considered’ rather than ‘we consider’.

We would like to acknowledge the contribution in the development of the Journal of members of the editorial board and leading scientists, professors Anatolii Sokolenko, Stanka Damyanova, Egon Schnitzler, Galyna Simakhina, Yurii Bilan, Jasmina Lukinac, Irina Ustinovich, Mark Shamtsyan and Mircea Oroian.

The publication of articles in our journal is free, as the publisher of the Journal, the National University Food Technologies (Kyiv, Ukraine), finds the necessary resources for functioning of the Journal and open access policy. We seek to expand the geography of authors and editorial board members, and invite the world scientific community to cooperate.

We believe that the high potential and creativity of the authors of the articles and the professionalism of the editorial scientific team will help maintain the high level of the Ukrainian Journal of Food Science and contribute to the authority of the Journal in professional communities.

We sincerely wish the authors, the editorial board and our readers good health, new scientific achievements and good publications!

Editor-in-Chief,
Prof. Viktor Stabnikov

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Effects of microwave and heat-moisture treatments on color characteristics, particle size and water distribution of potato starch

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Abstract

Introduction. The purpose of this study is to assess the effect of microwave and heat-moisture treatments on color characteristics, particle size and water distribution of potato starch.

Materials and methods. Native potato starch was modified by microwave and heat-moisture treatments. Such treatments of potato starch were done: single microwave treatment (MW); heat-moisture treatment assisted by microwave pre-treatment (MW-HMT), and heat-moisture treatment assisted by microwave post-treatment (HMT-MW).

Results and discussion. In general, HMT caused a slight increase of lightness (L* values), while single MW caused a slight decrease of lightness, indicating that the color of all the HMT treated samples became brighter and the color of the single MW treated sample (MWS) became darker. The color of MW-HMT samples became more reddish, while MWS, HMT and HMT-MW samples became more greenish. Although there were significant differences of the difference of color (ΔE), it could conclude that all treatments did not markedly change potato starch color for ΔE was always below 5. Median diameter (D50), particle diameter of volume (D(4,3)) and particle diameter of surface (D(3,2)) of all treated starch were higher compared with native starch (NS), while the value of specific surface area was significantly decreased by MW and HMT, indicating that these treatments can cause expansion, partial gelatinization and agglomeration of starch granules, resulting in large particle size of starch granules. MW and HMT treatments of potato starch caused the relaxation time T_{21} shifted toward faster relaxation times compared with native starch. Although the water in all starch samples was the main water which at least accounted for 90%, three peaks were observed in relaxation time T_2 of MW treated starch (MWS, MW-HMT and HMT-MW), and two peaks were observed in relaxation time T_2 of NS and HMT samples, which indicated the MW treated starch had three different state water, while NS and single HMT treated starch only had two different state water. Furthermore, MW and HMT treatments could change the water distribution and improve the interaction between starch and water.

Conclusions. Although MW and HMT treatments did not markedly change potato starch color, these treatments can cause expansion, partial gelatinization and agglomeration of starch granules, resulting in large particle size of starch granules. MW and HMT treatments could change the water distribution and improve the interaction between starch and water.

Introduction

Various modification methods have been used to enhance starch properties, including physical (Wang et al., 2018; Colussi et al.,2020), chemical (Ariyantoro et al.,2018) and enzymatic or combined ways (Li et al., 2019). Physical modifications are widely used for starch modification not only because of safety and simplicity, but also because of sustainability and environmental friendliness (Chandrasekaran et al., 2013; Kaur et al., 2012). Microwave treatment is widely used in the food industry at the working frequency of 915 MHz to 2450 MHz in most part of the world (Hoz et al., 2013). The microwave frequency, system composition, density, temperature and other factors influent the dielectric properties which are crucial indexes to evaluate the ability of food to absorb and transform microwave electromagnetic energy to thermal energy. The dielectric properties of starch-based materials are distinct due to their discrepant compositions, moisture content, metal ion concentration, porosity, and other parameters of a starch-based system (Tao et al.,2020). Recent researches show that microwave treatment can lead to changes in granule morphology, molecular chain structure and crystalline structure, which consequently affects its functional properties, such as solubility (Singh et al., 2012), swelling capacity (Deka and Sit, 2016), gelatinization (Oyeyinka et al.,2021), retrogradation (Chen et al., 2021) and digestion ability (Zeng et al., 2016). For example, microwave treatment decreased the swelling power, transparency and thermal properties of millet starch, destroyed the original appearance of the starch granules and formed smaller and lamellar gel blocks (Li et al., 2019). Heat- moisture treatment (HMT) is another commonly used physical treatment that involves the treatment of starch granules at low moisture content (<35%) for a certain time period (15 min-16 h) at relatively high temperatures (90 ◦C -120 ◦C) above glass transition temperature but below the gelatinisation temperature (Li et al., 2020; Chatpapamon et al., 2019) . Previous researches show that HMT promotes changes in X-ray diffraction (XRD) patterns, granule morphology, gelatinization properties, swelling power and enzyme digestibility (Chen et al., 2017; Zhou et al., 2020). Moreover, HMT improves the liquefaction of amylose and quality of recrystallization due to molecular chain arrangement inside the starch granule (Jiranuntakul et al., 2011).

Previous studies have reported the impacts of single microwave treatment and single heat-moisture treatment on morphological structural properties, thermal and physicochemical and digestibility of starch (Sui et al., 2015; Zhou et al., 2020). However, few studies exploring the effect of microwave treatment and heat-moisture treatment on the color characteristics, particle size and water distribution of potato starch have been reported.

Therefore, the purpose of this study is to assess the effect of microwave and heat-moisture treatments on color characteristics, particle size and water distribution of potato starch.

Materials and methods

Materials

Potato tubers locally known as Favorita cultivar were purchased from a local market in Hezhou city, China. Native starch from potato tubers was extracted with the technology of wet milling extraction as pervious method of Dhritiman Deka and Nandan Sit (Deka and Sit, 2016) and the dried starch was ground and passed through 80 mesh sieve and kept in airtight plastic containers for further analysis.

Starch samples with single and dual modification

Heat- moisture treatment. Heat-moisture treatment was performed according to the method of Deng (Deng et al., 2021) with some modifications. 70 g native starch powder (NS) was weighted in 500 mL Duran laboratory bottle, and ultrapure water was added to adjust moisture content to 25%. After thorough mixing, the glass containers were sealed and equilibrated for 24 hours at 25 °C, samples were then reacted in a hot-air oven (DH411C, Yamato Scientific Co.Ltd., Japan) at 90 °C for 1.5 h, 4 h, 8 h and 12 h respectively. After heat-moisture treatment, the starch samples were dried in a drying oven at 45 °C for 24 h to make sure the moisture content less than 12 %. The dried potato starch pulverized for 45 s using a universal pulverizer, passed through an 80-mesh sieve, vacuum-packed in polyethylene bags, and stored in an airtight container for conducting further studies. The prepared starches were named HMT1.5, HMT4, HMT8 and HMT12.

Microwave treatment. Adjustment of starch moisture content (25 %) and equilibration were carried out according to the method in Section 2.2.1. Then the starch was placed flat into a petri dish with a diameter of 18 cm, covered with microwave plastic film and evenly pricked 10 holes with toothpicks. The petri dish with starch was exposed in a microwave oven (Galanz, G80F20CN2L-B8(RO), Guangdong Galanz Microwave Appliance Manufacturing Co., LTD, China) to perform the microwave treatment with 400 W power for 5 min (increasing the power level or time of heating in microwave will cause the starch granules to burn). The treated starch sample was dried and stored as the method in Section 2.2.1. The prepared starches were named MWS.

Dual modification.

(1) Heat-moisture treatment assisted by pre- treatment of microwave (MW-HMT): The MWS sample was treated with heat-moisture treatment according to the method in Section 2.2.1 to obtain MW-HMT1.5, MW-HMT4, MW-HMT8 and MW-HMT12.

(2) Heat-moisture treatment assisted by poste-treatment of microwave (HMT-MW): HMT samples were treated with microwave according to the method in Section 2.2.2 to acquire HMT1.5-MW, HMT4-MW, HMT8-MW and HMT12-MW.

The treated samples were analyzed for physicochemical, structural and digestive properties. Untreated native potato starch (NS) was used as control.

Color characteristics

Color measurements of NS and treated starch were carried out using a colorimeter (CR-400, Konica Minolta Inc., Japan.) after the calibration of the equipment with a standard-white reflection plate. L^* indicates lightness, which varies from black (L^* =0) to white (L^* =100), a* is greenness / redness value, which varies from green (-60) to red (+60) and b* is blueness/yellowness value, which varies from blue (-60) to yellow (+60). Color difference (ΔE) between treated starch sample and the native starch (NS) was calculated with the equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

$$\Delta L^* = L^* - L_0^* \quad (2)$$

$$\Delta a^* = a^* - a_0^* \quad (3)$$

$$\Delta b^* = b^* - b_0^* \quad (4)$$

where L^*, L_0^* is the lightness of treated starch and NS; a^*, a_0^* is the greeness/ redness value of treated starch and NS; b^*, b_0^* is the blueness/yellowness value of treated starch and NS. The smaller value of ΔE indicates the smaller color difference between treated starch and NS.

Particle size distribution

A laser diffraction particle size analyzer ((BT-2001, Baxter Instruments Co. LTD, China) equipped with a dry dispersion unit was used to determine the particle size distribution of all the starch samples according to the manufacturer's instructions. The starch particle size parameters included particle diameter of volume (D(4,3)), particle diameter of surface (D(3,2)), specific surface area (S.S.A.), D50 represents the corresponding particle size which is smaller than 50% of the sample particles.

LF-NMR spin– spin relaxation (T_2) measurements

Low-field nuclear magnetic resonance (LF-NMR) spin-spin relaxation measurements were carried out using a Niumag Benchtop Pulsed NMR Analyzer (NMI120X, Niumag Electric Corp., Shanghai, China) to determine the water distribution of native and treated starch. A saturated $NaCl_2$ solution was used to equilibrate the water content of the samples for two weeks at 25 ◦C until the water activity of the samples (aw = 0.724) was constant and consistent according to the method of Fan (Fan et al., 2013). Approximately 1 g of equilibrated starch was placed into the NMR tube with diameter of 15 mm to measure T_2 using the Carr-Purcell-Meiboom-Gill (CPMG) sequence. The measurement temperature was 32 ± 0.1 °C and the proton resonance frequency was 18.0 MHz. Typical pulse parameters were as follows: the time of 90° plus (P_1) was 9.5 μs and the time of 180° plus (P_2) was 19.04 μs, the waiting time (TW) between subsequent scans was 3500 ms, data from 5000 echoes were acquired as 4 scan repetitions, and each measurement was performed at least 3 times.

Statistical analysis

All the experiments were conducted in triplicate unless otherwise stated. The Statistical analysis was performed on Data Processing System (version 7.05) and charts were done in Origin Pro 8. Data were analyzed using ANOVA with Duncan's multiple range test, and the values were considered significantly different when $p\leq0.05$.

Results and discussion

Color characteristics

The color values of the native potato starch (as control) and treated starch were showed in Table 1. In general, HMT caused a slight increase of lightness (L* values), while single MW treatment caused a slight, although significant, decrease of lightness, indicating that the color of all the HMT treated samples became brighter and the color of the single MW treated sample (MWS) became darker. The a* values of treated samples varied significantly in different ways depending on the treated ways. Although there was no significant difference of a* values in starch samples with the same treated method, the color of MW- HMT samples became more reddish, while MWS, HMT and HMT-MW samples became more greenish. As can be seen from Table 1, all the treated samples became more yellowish, as significant increase of b* values were obtained for treated samples. Although there were significant differences of the difference of color (ΔE), it could conclude that all treatments did not markedly change potato starch color from the analysis of ΔE, given that ΔE was always below 5, indicating that color differences was no visible differentiated of all the treated starch samples (Solaesa et al., 2021; García-Viguera and Zafrilla,2001).

Table 1

Color characteristics of native and treated potato starch samples

Samples	L^*	a^*	b^*	ΔE
NS	99.38±0.05^{cd}	4.80±0.01^{bcd}	-2.38±0.01^{i}	-
MWS	98.71±0.02^{e}	4.73±0.03^{g}	-2.21±0.03^{h}	0.70±0.02^{c}
MW-HMT1.5	99.31±0.24^{d}	4.81±0.03^{abc}	-2.02±0.06^{f}	0.42±0.05^{f}
MW-HMT4	99.46±0.20^{bcd}	4.84±0.02^{a}	-1.94±0.05^{e}	0.48±0.03^{e}
MW-HMT8	99.51±0.20^{abcd}	4.83±0.01^{ab}	-1.71±0.03^{bc}	0.70±0.02^{c}
MW-HMT12	99.54±0.06^{abcd}	4.80±0.03^{abcd}	-1.60±0.01^{a}	0.80±0.02^{a}
HMT1.5	99.38±0.07^{cd}	4.76±0.03^{defg}	-2.15±0.02^{g}	0.24±0.02^{g}
HMT4	99.59±0.02^{abc}	4.77±0.02^{cdef}	-2.03±0.02^{f}	0.41±0.01^{f}
HMT8	99.49±0.12^{abcd}	4.78±0.02^{cde}	-1.85±0.01^{d}	0.55±0.03^{d}
HMT12	99.66±0.08^{ab}	4.75±0.04^{efg}	-1.68±0.04^{b}	0.76±0.03^{ab}
HMT1.5-MW	99.70±0.05^{a}	4.74±0.01^{fg}	-2.14±0.01^{g}	0.41±0.03^{f}
HMT4-MW	99.50±0.05^{abcd}	4.75±0.01^{efg}	-1.85±0.01^{d}	0.55±0.02^{d}
HMT8-MW	99.68±0.13^{ab}	4.77±0.01^{defg}	-1.89±0.01^{d}	0.58±0.06^{d}
HMT12-MW	99.70±0.11^{a}	4.77±0.03^{def}	-1.74±0.02^{c}	0.72±0.05^{bc}

Note: all values are the mean of triplicate determinations ± SD. The means within the same column with different letters are significantly different ($P<0.05$).

Particle size distribution

The variation of particle size could reflect the changes in agglomeration of the starch before and after modification. The particle size distribution parameters of native and treated potato starch were showed in Table 2.

Table 2

Particle size distribution of native and treated potato starch samples

Samples	D50(μm)	D(4,3) (μm)	D(3,2) (μm)	S.S.A.(m^2/kg)
NS	34.12±0.40^{c}	36.60±0.38^{f}	22.24±0.56^{h}	99.93±2.54^{a}
MWS	34.29±0.16^{bc}	36.97±0.07cdef	24.06±0.07^{g}	92.35±0.26^{b}
MW-HMT1.5	34.38±0.09^{bc}	37.24±0.21^{bcdef}	29.67±0.12^{def}	74.87±0.32^{cd}
MW-HMT4	34.53±0.03^{abc}	37.35±0.08^{abcde}	30.51±0.02^{abc}	72.83±0.06^{de}
MW-HMT8	34.91±0.27^{ab}	37.79±0.26^{ab}	30.88±0.23a	71.96±0.53^{e}
MW-HMT12	35.14±0.68^{a}	37.95±0.65a	30.76±0.56^{ab}	72.24±1.31^{e}
HMT1.5	34.61±0.28^{abc}	37.48±0.19^{abcd}	30.28±0.17^{abcd}	73.38±0.41^{cde}
HMT4	34.82±0.21^{ab}	37.52±0.22^{abc}	29.51±0.23ef	75.29±0.59^{c}
HMT8	34.73±0.49^{abc}	37.33±0.67^{abcde}	29.35±0.25^{f}	75.71±0.65^{c}
HMT12	34.64±0.23^{abc}	37.42±0.17^{abcde}	29.96±0.44^{cdef}	74.18±1.08^{cde}
HMT1.5-MW	34.36±0.13^{bc}	37.03±0.18^{cdef}	23.97±0.42g	92.72±1.60^{b}
HMT4-MW	34.29±0.19^{bc}	37.00±0.07cdef	29.41±0.24^{ef}	75.55±0.62^{c}
HMT8-MW	34.32±0.11^{bc}	36.82±0.09^{def}	30.11±0.23^{bcde}	73.63±0.84^{cde}
HMT12-MW	34.54±0.07^{abc}	36.75±0.09^{ef}	30.27±0.46^{abcd}	73.41±1.10^{cde}

Notes: all values are the mean of triplicate determinations ± SD. The means within the same column with different letters are significantly different ($P<0.05$).

The D50, D(4,3) and D(3,2) of all treated starch were higher than NS, while the value of S.S.A. was significantly decreased by MW and HMT, which was agreed with precious studies about HMT treated lily starch (Li et al., 2020) MW treated waxy hull-less barley starch (Chen et al., 2021). The reason for the large particle size of MW and HMT modified starch was that the internal temperature of the granules rose rapidly during the MW and HMT treatment, and the internal pressure increased, causing the starch granules to expand, resulting in partial gelatinization and agglomeration of the granules (Li et al., 2020; Chen et al., 2021) .

Relaxation time (T_2) analysis

In starch-based foods, the physical properties of water can significantly affect the processing quality of starch. LF-NMR technology is the most effective technology for evaluating the distribution and state of water in the starch-water system. Relaxation time (T_2) is the time required for an excited spin-spin proton to reach dynamic equilibrium after energy exchange with adjacent protons, reflects the difference in the degrees of freedom of water. The less hydrogen protons are bound or higher degree of freedom is associated with longer transverse relaxation time (Han et al., 2009), while the more hydrogen protons are bound or lower degree of freedom corresponds to a shorter transverse relaxation time (Pitombo and Lima, 2003; Luo et al., 2020).

As shown in Figure 1-3 and Table 3, three peaks were observed in T_2 of MW treated starch (MWS, MW-HMT and HMT-MW), while two peaks were observed in T_2 of NS and HMT samples. The first peak T_{21} (0.01-1 ms) corresponded to bound water, which interacted with the surface of amylose and amylopectin chains (Tang et al., 2000).

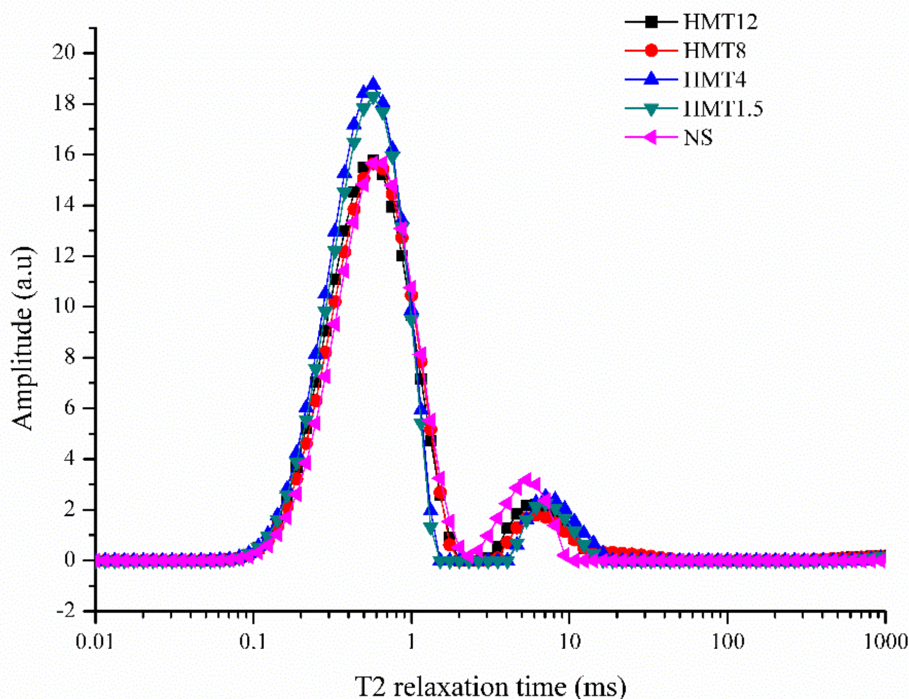

Figure 1. Water distribution in native and single HMT treated starch by LF-NMR

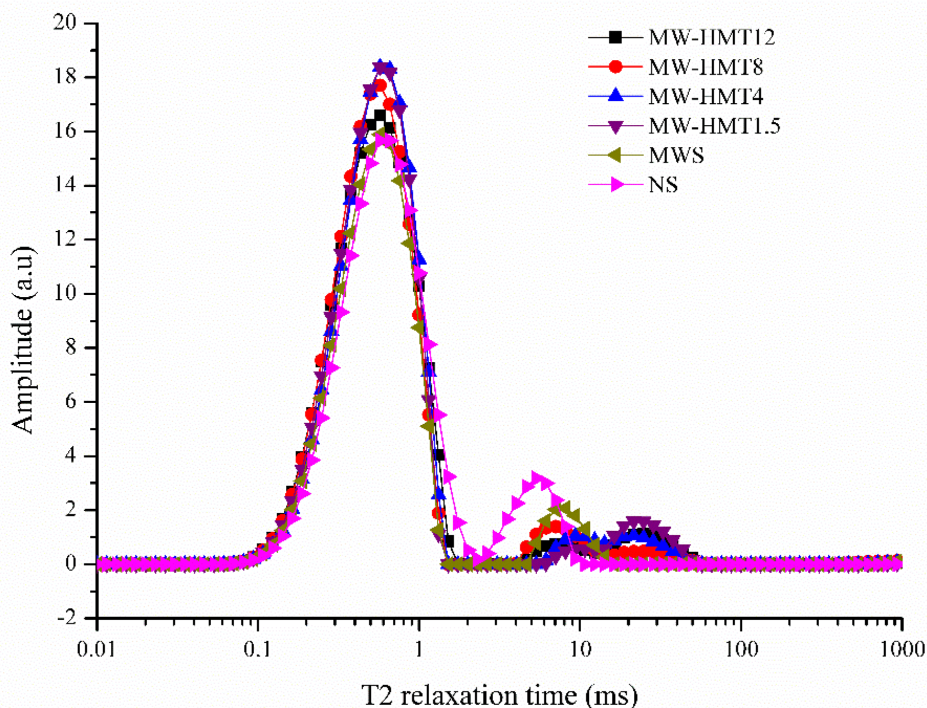

Figure 2. Water distribution in native and MW-HMT treated starch by LF-NMR

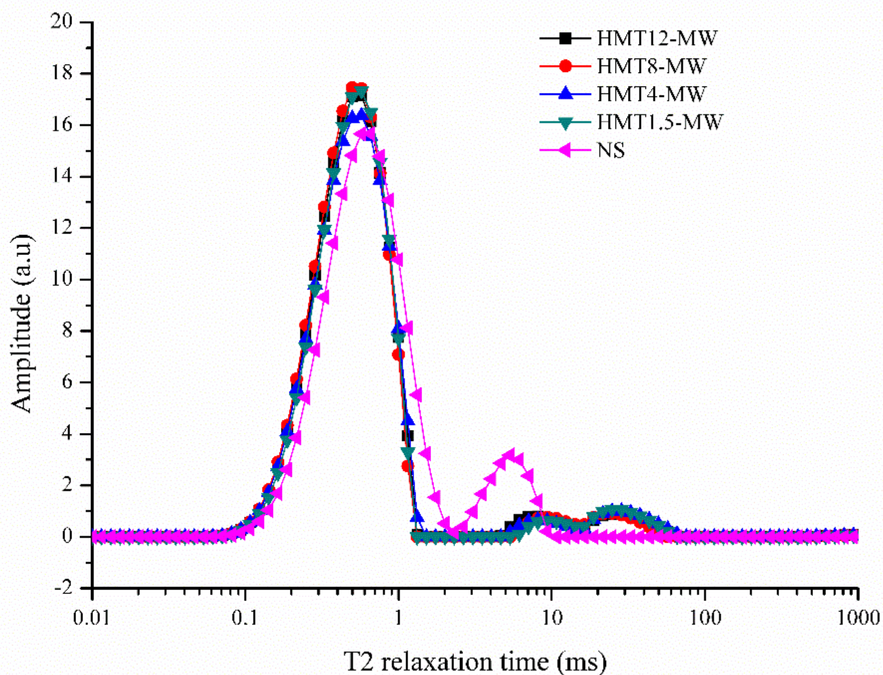

Figure 3. Water distribution in native and HMT-MW treated starch by LF-NMR

Table 3

Relaxation times (T_2) and corresponding peak areas percentages of water from native and treated starch

Samples	Relaxation Time (ms)			Proportion of water in different state (%)		
	T_{21}	T_{22}	T_{23}	PT_{21}	PT_{22}	PT_{23}
NS	0.62 ±0.06[a]	5.74 ±0.57[b]	-	90.48 ±0.98[c]	9.52 ±0.98[a]	-
MWS	0.59 ±0.01[ab]	8.43 ±3.24[ab]	30.61 ±3.02[a]	92.56 ±0.80[ab]	1.14 ±0.06[e]	6.29 ±0.74[a]
MW-HMT1.5	0.57 ±0.00[ab]	7.73 ±2.26[ab]	25.01 ±4.90[a]	93.06 ±0.04[ab]	1.58 ±0.39[de]	5.36 ±0.35[ab]
MW-HMT4	0.60 ±0.05[ab]	8.52 ±0.70[ab]	24.71 ±2.74[b]	93.39 ±0.52[ab]	3.92 ±0.88[c]	2.69 ±0.72[c]
MW-HMT8	0.57 ±0.00[ab]	7.60 ±0.65[ab]	24.66 ±0.16[a]	93.11 ±0.75[ab]	3.57 ±1.42[c]	3.33 ±2.17[bc]
MW-HMT12	0.57 ±0.00[ab]	8.06 ±0.00[ab]	25.76 ±1.40[a]	93.57 ±0.30[ab]	2.85 ±0.64[cd]	3.57 ±0.94[bc]
HMT1.5	0.57 ±0.00[ab]	7.10 ±0.99[ab]	-	93.32 ±0.18[ab]	6.68 ±0.18[b]	-
HMT4	0.57 ±0.00[ab]	6.75 ±0.53[ab]	-	92.59 ±0.26[ab]	7.41 ±0.26[b]	-
HMT8	0.57 ±0.00[ab]	6.44 ±0.53[ab]	-	93.31 ±1.33[ab]	6.69 ±1.33[b]	-
HMT12	0.57 ±0.00[ab]	6.60 ±0.65[ab]	-	92.37 ±0.94[b]	7.63 ±0.94[b]	-
HMT1.5-MW	0.57 ±0.00[ab]	8.72 ±0.86[a]	24.77 ±0.00[a]	93.61 ±0.29[ab]	2.32 ±0.26[cde]	4.07 ±0.04[abc]
HMT4-MW	0.54 ±0.05[b]	8.72 ±0.86[a]	28.61 ±0.18[a]	93.97 ±0.53[a]	2.99 ±1.13[cd]	3.05 ±1.66bc
HMT8-MW	0.54 ±0.05[b]	7.73 ±2.26[ab]	28.76 ±5.64[a]	93.77 ±0.88[ab]	2.52 ±0.13[cde]	3.71 ±1.01[bc]
HMT12-MW	0.54 ±0.04[b]	8.52 ±0.70[ab]	27.33 ±2.22[a]	93.77 ±0.14[ab]	3.19 ±0.40[cd]	3.04 ±0.41[bc]

Notes: all values are the mean of triplicate determinations ± SD. The means within the same column with different letters are significantly different ($P<0.05$). – indicates that there is no value.

It can be seen from Table 3 that the MW and HMT treatments of potato starch caused the T_{21} shifted toward faster relaxation times compared with native starch (NS), indicating that MW and HMT treatments enhanced the starch-water interaction and self- binding of starch, and consequently resulting in lower mobility of water protons. The second peak T_{22} (1-20 ms) corresponded to "semi-crystalline lamellae water" (Tang et al., 2000), which existed in the narrow space between crystals. Due to different spaces, the water in them exhibited different mobilities. Peak of NS was closer to the left than treated starch; they were followed by the single HMT treated starch (HMT1.5, HMT4, HMT8 and HMT12). The peaks T_{22} of MW treated starch were close to the right and connected with its next peak. The third peak T_{23} was located in T_2 between 20 ms and 100 ms was attributed to immobilized water, which was regarded as water inside the hexagonal channels of B-type crystal clusters and was defined as "channel water in B-type crystal" by a previous study (Chen et al., 2019). It can be observed from Figure1, the native and HMT treated starch did not have channel water in B-type crystal, while all the MW treated starch contained a certain amount of channel water in B-type crystal.

The proportion of different state water in native and treated starch were summarized in Table 3. The areas under different component represented the proportion of different state water distribution, namely PT_{21} (bound water), PT_{22} (semi-crystalline lamellae water), PT_{23} (channel water in B-type crystal), respectively. Although the water in all starch samples was the main water which at least accounted for 90%, the MW treated starch had three different state water, NS and single HMT treated starch only had two different state water. There were significant differences of PT_{21} and PT_{22} between NS and all treated starch, NS had the lowest PT_{21} but highest PT_{22}, indicating MW and HMT treatments could change the water distribution and improve the interaction between starch and water. No significant differences of PT_{21} were observed between MW treated starch and single HMT treated, but all MW treated starch had lower PT_{22} than single HMT treated starch; furthermore, all the MW treated starch contained a certain amount of channel water in B-type crystal (PT_{23}). These results indicated that MW treatment could result in a certain level of semi-crystalline lamellae water shifting to channel water in B-type crystal.

Conclusions

In this study, different treated potato starch samples were prepared with MW and HMT methods, and the color characteristics, particle size and water distribution of native and treated potato starch were evaluated by using colorimeter, laser diffraction particle size analyzer and LF-NMR. Although color differences was no visibly differentiated between NS and all treated starch, HMT treatment caused a slight increase of lightness (L* values), while single MW treatment caused a slight decrease of lightness, indicating that the color of all the HMT treated samples (HMT, MW-HMT, HMT-MW) became brighter and the color of the single MW treated sample (MWS) became darker. Although there were significant differences of the differences of color (ΔE), it could conclude that all treatments did not markedly change potato starch color for ΔE was always below 5, indicating that color difference was no visible differentiated of all the treated starch samples. The results of article size distribution showed that D50, D(4,3) and D(3,2) of all treated starch were higher than NS, while the value of S.S.A. was significantly decreased by MW and HMT, indicating that MW and HMT treatments can caused expansion, partial gelatinization and agglomeration of starch granules, resulting in large particle size of starch granules. Although the water in all starch samples was the main water which at least accounted for 90%, three peaks were observed in T_2 of MW treated starch (MWS, MW-HMT and HMT-MW), two peaks were observed in T_2 of native and single HMT treated starch, which indicated the MW treated starch had three different state water, while NS and single HMT treated starch only had two different state water. There were significant differences of PT_{21} and PT_{22} between NS and all treated starch, NS had the lowest PT_{21} but highest PT_{22}, indicating MW and HMT treatments could change the water distribution and improve the interaction between starch and water.

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Partial purification and characterisation of polyphenol oxidase from faba bean (*Vicia faba*) coat

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Abstract

Introduction. The aim of this study is to isolate polyphenol oxidase (PPO) enzyme from faba bean coat, and to determine its optimum pH and temperature, K_m and V_{max} parameters, to investigate biochemical properties such as pH and temperature stability, substrate selectivity, storage stability of enzyme, and also effects of some metals and inhibitors on its activity.

Materials and methods. The protein precipitation was done by using ammonium sulfate salt and then, dialysis was performed to remove salt ions after precipitation. Protein content of the samples at each step was determined according to Bradford method, and enzymatic activity was determined spectrophotometrically.

Results and discussion. The properties of PPO enzyme, which was purified 3.1-fold, were determined using catechol as substrate. K_m and V_{max} values of the enzyme were determined to be 5.53 mM and 4424,58 $U.ml^{-1}min^{-1}$, respectively. The enzyme showed the highest activity at pH 5.0 and 10 ºC. It saved about 60% of its activity after a 30 min incubation period at its optimum pH. Substrate specificity of PPO was studied using eight substrates including pyrogallol and 4-methylcatechol. When the thermal stability profile was examined in the 10–60 ºC temperature range, it exhibited more than 50% of its activity in the range of 10–40 ºC after 1 hour of incubation. While L-ascorbic acid, sodium sulfite and L-cysteine showed strong inhibitory effects, the studied metals showed variable effects on the enzyme activity.

Conclusions. The polyphenol oxidase enzyme activity causes undesirable changes and nutritional value loss in some fruits and vegetables. The results obtained provide information about faba bean PPO and give clues to the effective methods for controlling browning during storage.

Introduction

The Fabaceae, also known as the Leguminosae, is a large, economically and medicinally important family. Because natural nitrogen fixation is performed by *Rhizobium* bacteria, which can be found in the root nodules of leguminous plants. Moreover, the dietary consumption of legumes is associated with lower incidence of chronic degenerative diseases (Turco et al., 2016). Broad bean or faba bean (*Vicia faba*) is a one-year plant from the Fabaceae family and faba beans used as a vegetable which consumed fresh and green or dried. This plant has an important place in diets of Chinese, English, Turkish and Mediterranean because of a useful source of polyphenols (Turco et al., 2016). Faba bean has L-DOPA (L-3,4-dihydroxyphenylalanine) content (Topal and Bozoglu, 2016; Mohseni and Golshani, 2013) which medically used in the treatment of Parkinson's disease. In the technology, it and some other Fabaceae species is used in the production of L-DOPA (Goyoaga et al., 2008). However, faba beans are also used as additives in animal feeds due to their high protein content. Field bean (*Vicia faba* var minor) especially is a good protein source for animal feedstuffs (Antongiovanni et al., 2002; Perella et al., 2009). Because of the symbiosis with nodule-forming bacteria (Rizobium), legumes with high nitrogen fixation have an important place in agriculture as "green manure" (Ayaz and Sokmen, 2015). In the study carried out on the use of faba bean as a green manure in broccoli cultivation, it was observed that increased broccoli yield and some properties (Yilmaz and Sahin, 2014).

In general, the chemical composition of plants is significantly different due to some factors that cover types of seed (genetic), age, and environment, such as soil conditions and climate, as well as weather and growing conditions, such as fertilization, pesticides and pests. One of the important chemical components of plants is the PPO enzyme. It is found in different parts of plants including roots, leaves, flowers and vascular tissues (Constabel and Barbehenn, 2008). PPO catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones in the presence of molecular oxygen. Copper is required as a prosthetic group to its activity. There are three classes of enzymes belonging to the PPOs: catechol oxidase (EC 1.10.3.1; 1,2-benzenediol: oxygen oxidoreductase), laccase (EC 1.10.3.2; *p*-benzenediol: oxygenoxidoreductase) and tyrosinase (EC 1.14.18.1; monophenol monooxygenase, cresolase) (NC-IUBMB, 2019). PPOs are commonly found in the plant kingdom and are a major cause of browning during the harvesting and storage of many fruits and vegetables. In the other hand, the oxidative enzyme levels including PPO are affected by plant-insect interactions. When plants are injured by insects, the reactive oxygen species are overproduced and the plants overcome this oxidative stress by producing an efficient enzymatic antioxidant defense system (Nabity et al., 2006; Chen et al., 2009; He et al., 2011; Panadare and Rathod, 2018). Therefore polyphenol oxidase is both involved in oxidation phenolic and plant defense system.

The faba bean coat, planted in Thrace region, is currently growing in popularity due to their premium texture and taste. However, there are no reports on the polyphenol oxidase from these cultivars, and also on pod (outer shell) of the plant. Therefore, the aim of this research was to isolate and characterize the biochemical properties of PPO from faba bean coat grown in Turkey. Although the studies on the property of PPO from various vegetables and fruits have also been carried out in the past, in the literature no studies have been conducted on broad bean coat except few studies on the isolation and characterization of PPO from broad bean leaf.

Material and method

Plant material and isolation of enzyme

Faba bean that planted at Edirne province in Turkey was purchased from a local market. The plants were washed and the beans were removed from their coats. The coats (outer shell) were used for enzyme extraction. Three hundred gram fresh coats were homogenized in 0.1 M phosphate buffer (pH 6.0) containing polyvinyl pyrolidone and Triton X-100 for 1 min using Waring blender at room temperature. Then the homogenate was filtered through cheesecloth. The filtrate was centrifuged (Heraeus Biofuge Stratos, Osterode-Germany) at 10,000 rpm and +4 °C for 20 min to remove plant residues. The supernatant was called "crude extract" and used as enzyme source for further study.

Partial purification of polyphenol oxidase

The crude extract of polyphenol oxidase enzyme was precipitated by slowly adding solid ammonium sulphate for 30% saturation at 4 °C into it. The protein precipitated was collected by centrifugation at 10,000 rpm for 45 min at 4 °C. Supernatant was used for further fractionation at between 30-65% and 65–80% ammonium sulphate saturation. Protein precipitate obtained at each step were dissolved in 0.1 M phosphate buffer (pH 6.0) and, enzymatic activity and protein determination were performed. The fraction precipitated between 30 and 65% saturation was showed highest polyphenol oxidase activity. This fraction was dissolved in a small amount of 0.1 M phosphate buffer (pH 6.0), and dialyzed overnight at 4°C in the same buffer on a magnetic stirrer. The dialyzed extract was used as partially purified enzyme source in the experiments.

Assays of enzyme activity and protein determination

In assay of PPO activity, the 0.1 ml enzyme solution was mixed with 2.9 ml catechol in 0.1 M phosphate buffer (pH 6.0) and the increases in absorbance were followed at 420 nm spectrophotometrically (Thermo Scientific Multiscan Go, Vantaa-Finland). Because the dialysate has colour, a sample blank consisting of enzyme and buffer solutions was prepared for each tube. One unit of polyphenol oxidase activity was defined as the amount of enzyme producing a 0.001 absorbance increase in sample per minute under the standard assay conditions (Soffan et al., 2014).

Protein content of the samples at each step was determined according to Bradford method using bovine serum albumin as a standard (Bradford, 1976).

Determination of optimum pH and temperature

PPO activity was determined in a pH range of 3.0–7.0 in 0.1 M citric acid buffer and 5.0–8.0 in 0.1 M phosphate buffer. The activity was carried out using the standard reaction conditions but changing the buffer and, the catechol was used as substrate. The optimum pH obtained for PPO was used in all other studies.

The activity of PPO was determined at temperatures ranging from 10 to 60 °C. The catechol substrate and citrate buffer (pH 5.0) were incubated in the appropriate medium until the selected temperatures were reached. After equilibration of the reaction mixture at the selected temperature, the enzyme solution was added and, the enzyme activity was determined. PPO activity was calculated as percent residual PPO activity at the optimum temperature.

Substrate specificity and enzyme kinetics

The substrate specificity of enzyme was investigated by using various phenolic compounds including catechol, 4-methyl catechol, pyrogallol, L-DOPA, gallic acid, chlorogenic acid, 4-methylcatechol, resorcinol and hydroquinone. The substrate solutions were prepared at 0.02 M concentration in citrate buffer (0.1 M, pH = 5.0) and enzyme activity was determined for each substrate. The relative activities (%) of each substrate tested were calculated according to the PPO activity of the catechol.

The kinetic constants, optimum pH and optimum temperature values of the substrates with high activity were determined. The K_m and V_{max} constants were determined from a Lineweaver–Burk plot under the optimum pH and temperature conditions. The substrates of catechol, 4-methylcatechol and pyrogallol were used at concentrations 5-50 mM, 10-30 mM and 10-30 mM, respectively.

pH stability

The PPO enzyme obtained from coats was pre-incubated for 30 minutes and 60 minutes in citrate buffer (0.1 M at pH 5.0, 5.5, 6.0, 6.5, 7.0). Then residual activity was determined using catechol substrate under the optimal conditions. Enzyme activity was performed under optimum conditions without incubation, and the residual activity of incubated enzyme expressed as relative activity (%).

Thermal stability

Enzyme solution was preheated (Wisebath Daihan Shaking Water Bath, Korea) for 15, 30, 45 and 60 min at 10–60 °C. The aliquot of enzyme solution was taken in test tubes and the tubes were cooled in an ice bath. The remaining activity of the enzyme was estimated under standard assay conditions in each case using catechol substrate. PPO activity determined under optimum conditions without waiting for temperature and it was used as the reference. The activity was defined as the percentage of the maximum activity level.

Determination of the effect of metal ions and some chemicals

The effects of various inhibitors such as L-ascorbic acid, ethylenediamine tetraacetic acid (EDTA), sodium azide, L-cysteine, potassium cyanide and sodium bisulfite on PPO activity were examined by incubating a mixture consisting of the enzyme solution, buffer solution and inhibitors (at 3 mM and 6 mM concentrations,) for 5 min at room temperature. Enzyme activity without any reagent was then assayed under standard conditions. Enzymatic activities were expressed as relative values with the activity of the enzyme without any reagent.

The metal ion solutions (Cu^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}) were prepared at 1 mM and 10 mM concentrations. To research for each metal effect, activity measurements were done under standard conditions using catechol substrate. Enzymatic activity was expressed as relative values (%) with reference to the activity of the enzyme without any metal ions.

Results and discussion

PPO isolation and characterization studies are performed from various sources in order to determine the properties of the enzyme. In the study PPO isolation was made from faba bean coats, and the crude extract was partially purified by applying ammonium sulphate precipitation and dialysis. Proteins precipitated at 30–65% salt concentration showed the highest PPO activity and, the precipitate was dialysed by the buffer. While the total specific activity of the enzyme in the homogenate was 2072.4 U, the total specific activity of the partially purified PPO was 6415.8 U. According to these results, the enzyme was purified 3.1-fold for the faba bean coat. The enzyme purification steps are summarized in Table 1. In the studies about Fabaceae family, it was reported that PPO was purified 3.5-fold for green bean (*Phaseolus vulgaris* L.) coat and 3.7-fold for green bean pod after 60% salt precipitation and dialysis (Guo et al., 2009), and 2.1 times from field bean (*Dolichos lablab* var. lignosus) seeds after 40-80% salt precipitation (Paul and Gowda, 2000).

Table 1

Steps for polyphenol oxidase from faba bean coat

Partially purification steps	Volume [ml]	Protein [$mg.ml^{-1}$]	Activity [$U.ml^{-1}$]	Specific Activity [$U.mg^{-1}$ prt]	Total Specific Activity [U]	Purification fold
Crude extract	660	11.8	37	3.14	2072.4	1
$(NH_4)_2SO_4$ (0-30%)	-	3.75	20	5.33	-	-
$(NH_4)_2SO_4$ (30-65%)	-	10.05	78	7.76	-	-
Dialysate	51	20.4	2568	125.8	6415.8	3.1

pH is one of the most important factors that influence enzyme activity. PPO optima for faba bean coats was studied using the catechol substrate in two different buffer systems the pH range among 4.0-8.0. Optimum pH was determined as pH 6.0 with phosphate buffer and pH 5.0 with citrate buffer (Figure 1). For the pyrogallol, 4-methylcatechol and hydroquinone substrates, the optimum pH was found to be 5.0 in citrate buffer.

To predict the behaviour of the enzymes, the biochemical characterization of them is searched in the matter of pH optimum and stability, thermal activation and stability, substrate specificities, inhibitors or activators. pH is one of the most important factors that influence enzyme activity. Although the optimum pH of the PPO enzyme varies depending on the plant source, it is seen that it is generally in the range of 4.0-8.0. Different optimum pH values for PPO obtained from different sources are reported in the literature. It was reported as 6.8-7.2 for green beans (*Phaseolus vulgaris* L.) (Guo et al., 2009), 4.0 for field bean (*Dolichos lablab*) seeds (Paul and Gowda, 2000), 6.0 for mung bean leaf (Shin et al., 1997) and 9.0 for soybean (Nagai and Suzuki, 2003) using catechol as substrate.

To determine the effect of temperature on faba bean PPO, enzymatic activity determination was performed at different temperatures. Differences in temperature can affect the activity by changing the solubility of oxygen necessary for the PPO activity or causing changes in the conformation of the enzyme. In the literature, it is seen that the optimum temperature values for PPO generally change in the range of at 10-50 °C. In the study, the activity of PPO was measured at temperatures among 10-60 °C for catechol (Figure 2), 4-

methylcatechol and pyrogallol substrates at optimum pH. The enzyme showed the highest activity at 10 ºC for all substrates, but the studies were carried out at 20 ºC where the next highest activity was observed due to the difficulty of maintaining this temperature (10 ºC) as stable. Using catechol as substrate, it has been reported as being at 20-30 °C for green beans (*Phaseolus vulgaris* L.) (Guo et al., 2009), 40 °C for soybean sprouts (Nagai and Suzuki, 2003), 10 °C for borage plant (*Trachystemon orientalis* L.) (Alici and Arabaci, 2016), 30 °C for eggplant (Ng and Wong, 2015), 35 °C for blueberry (*Vaccinium corymbosum* L.) (Siddiq and Dolan, 2017), 25 °C for grape (Onez, 2006) and plums (*Prunus domestica*) (Ionita et al., 2017).

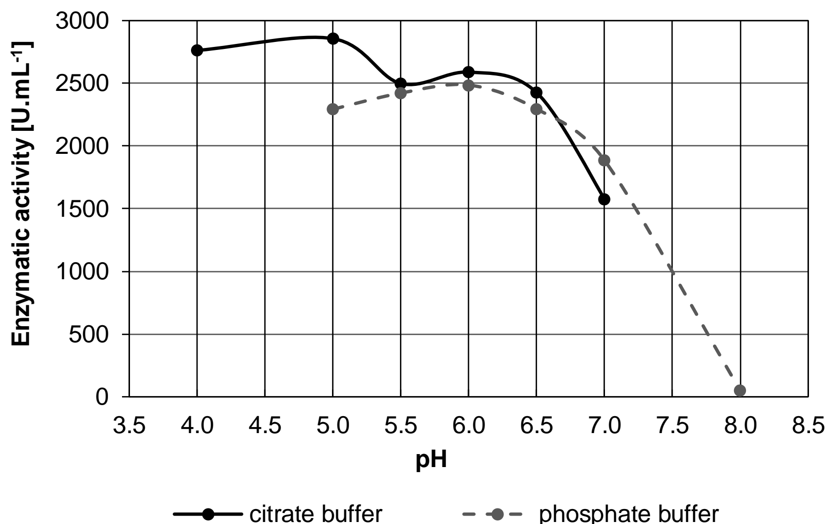

Figure 1. Effect of pH on PPO activity from faba bean coat

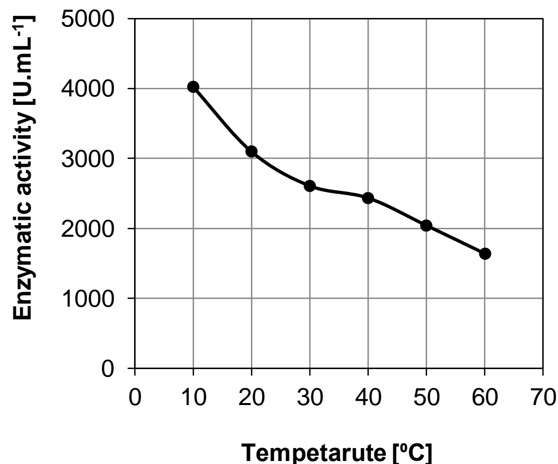

Figure 2. Effect of temperature on PPO activity from faba bean coat

K_m and V_{max} values for PPO from faba bean coat for three phenolic compounds which generally use as substrates for polyphenol oxidase are presented in Table 2. From the V_{max}/K_m ratio the best substrates for PPO are catechol and pyrogallol. Paul and Gowda (2000) reported these K_m values for field bean (*Dolichos lablab* var. lignosus) PPO; 1.18 mM for L-DOPA, 4.0 mM for 4-methylcatechol, 10.5 mM for catechol and 12.5 mM for pyrogallol. In another study carried out by Shin *et al.* (1997), K_m values for mung bean leaf PPO were found to be 4 mM for 4-methylcatechol and 24 mM for L-DOPA. But K_m value for green bean (*Phaseolus vulgaris* L.) coat PPOIa and PPOIb was calculated 7.6–4.1 mM, 10.6–10.7 mM, 14.5–15.8 mM and 35.2–34.7 mM for pyrogallol, catechol, 4-methylcatechol and L-DOPA, respectively (Guo et al., 2009). According to literature, the interest of PPOs from various plant sources for various substrate varies widely.

Table 2

Kinetic parameters of PPO from faba bean coat

Substrate	K_m [mM]	V_{max} [U.mL^{-1} dk^{-1}]	V_{max}/K_m
Catechol	5.53	4424.6	800.1
Pyrogallol	3.05	2263.1	741.9
4-methylcatechol	12.18	2278.3	264.3

PPO substrate specificity studied using eight well known substrates. Catechol showed maximum activity (3370±135.4 U.ml^{-1}) and this was considered as 100% to compare its activity with other substrates (Table 3). The existence of hydroxyl groups in the case of catechol (-OH at ortho position, as in pyrogallol and 4-methylcatechol), the activity increased. By contrast, the substrates without catechol ring (-OH at meta or para positions) and with a functional group at other positions of the ring showed very less of activity. Pyrogallol and 4-methylcatechol were best substrates after the catechol. Optimum pH and optimum temperature study was performed for these substrates. For both substrates, the optimum pH was determined as 5.0 and the optimum temperature was 10 °C as determined in the catechol substrate. Other Leguminosae PPO enzymes studied by the other researchers also showed high activity against catechol, 4-methylcatechol, pyrogallol and L-DOPA substrates (Shin et al., 1997; Paul and Gowda, 2000; Nagai and Suzuki, 2003; Guo et al., 2009).

Table 3

Substrate specificity of PPO from faba bean coat

Substrate	Relative activity [%][a]	Structural specificity of the substrate
Catechol	100	Ortho-dihydroxy (1,2-OH)
Pyrogallol	81.8±3.3	Trihydroxy (1,2,3-OH)
4-Methylcatechol	77.2±0.6	Ortho-dihydroxy (1,2-OH, 4-CH_3)
Hydroquinone	38.9±0.1	Para-dihydroxy (1,4-OH)
L-DOPA	33.5±1.9	Orto-dihydroxy (1,2-OH, 4-X) [X=CH_2-CH(NH_2)-COOH]
Resorcin	24.8±0.8	Meta-dihydroxy (1,3-OH)
Chlorogenic acid	20.9±2.8	Ortho-dihydroxy (1,2-OH)
Gallic acid	14.4±1.7	Trihydroxy (1,2,3-OH, 5-COOH)

[a]Each value represents the mean±SD (n=3)

For determine to pH stability, the enzyme solution was incubated in citrate buffer solutions ranging from 5.0 to 8.0 at 4 °C after 30 min and 60 min incubation period. The remaining enzyme activity was measured using catechol as substrate. At the end of the 60 minutes incubation period the highest activity was determined in the pH 5.0 buffer that is the optimum pH of the enzyme, and the most activity loss was determined in pH 7.0 buffer. As seen as in Figure 3, the remaining activity at pH 5.0 was 56%, while at pH 6 it was 41%. The enzyme is not stable at pH 8.0. In the pH stability studies for PPO enzyme isolated from various sources, it was determined that they all showed the highest activity in buffers at their optimum pH (Mdluli, 2005; Gao et al., 2009; Kuyumcu, 2014).

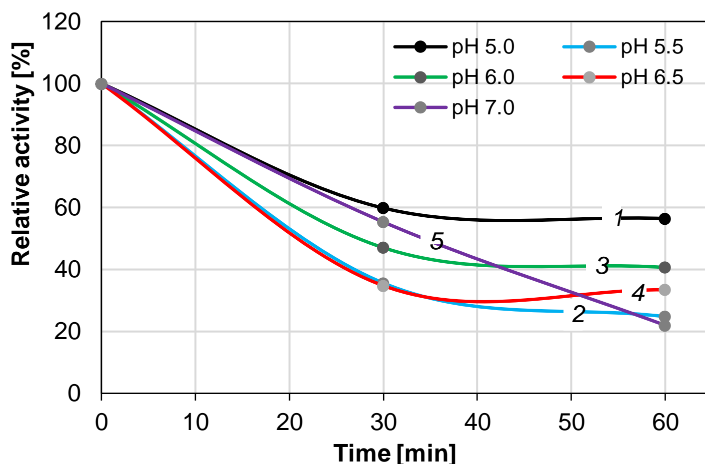

Figure 3. Effect of pH on stability of PPO activity (The residual activity of incubated enzyme expressed as% relative activity):
1 – pH 5.0; 2 – pH 5.5; 3 – pH 6.0; 4 – pH 6.5; 5 – pH 7.0

Thermal inactivation of faba bean coat PPO was studied in the range 10-60 °C, for different time periods (15, 30, 45 and 30 min) as seen as Figure 4. The enzyme was preserved its activity at 90% at 10 °C which was determined as the optimum temperature, and at 85% at 20 °C where experimental studies were conducted. As stated in the study of Kuyumcu (2014) for mushroom PPO, after the incubations at optimum temperatures, the faba bean coat PPO has also substantially maintained its activity. The activity was maintained at 70% at 30 °C and 58% at 40 °C within the first 30 minutes, while enzyme activity decreased by half in the first 15 minutes at 50-60 °C (Figure 4). Therefore, it can be said the enzyme relatively thermostable. The results about the thermostability is in agreement with the results of the PPO study isolated from borage plant (Alici and Arabaci, 2016), quince (Yagar, 2004) and mulberry (Arslan et al., 2004). These results suggest that short incubation times could be worked at higher temperatures while longer incubation times should be chosen at low temperatures for food processes.

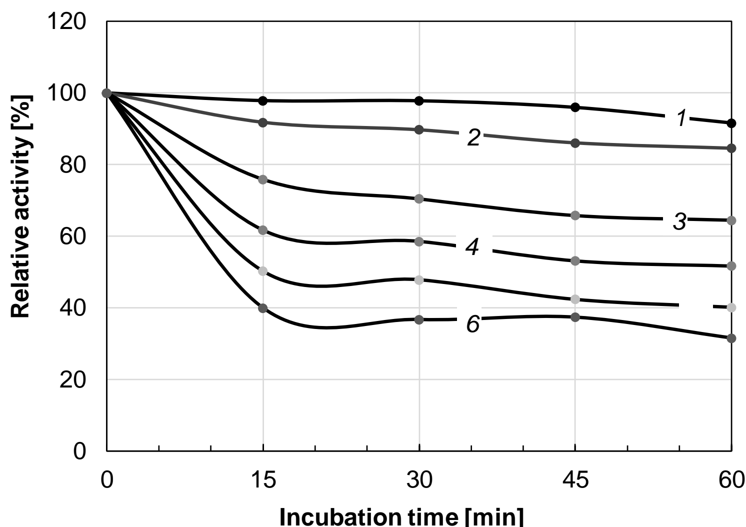

Figure 4. Effect of thermal treatments on PPO activity (The residual activity of incubated enzyme expressed as% relative activity.)
Temperature, °C: 1 – 10; 2 – 20; 3 – 30; 4 – 40; 5 – 50; 6 – 60.

In the study of ionic strength, it was observed that other metals, except the iron and cobalt ions, did not cause a major change in enzyme activity. While iron ion (74.1±2.8% at 10 mM and 92.4±2.4% at 1 mM concentrations) and cobalt ion (72.1±1.8% at 1 mM concentration) caused a decrease in enzyme activity, cobalt ion at 10 mM concentration caused an increase of 10% in enzyme activity (Figure 5).

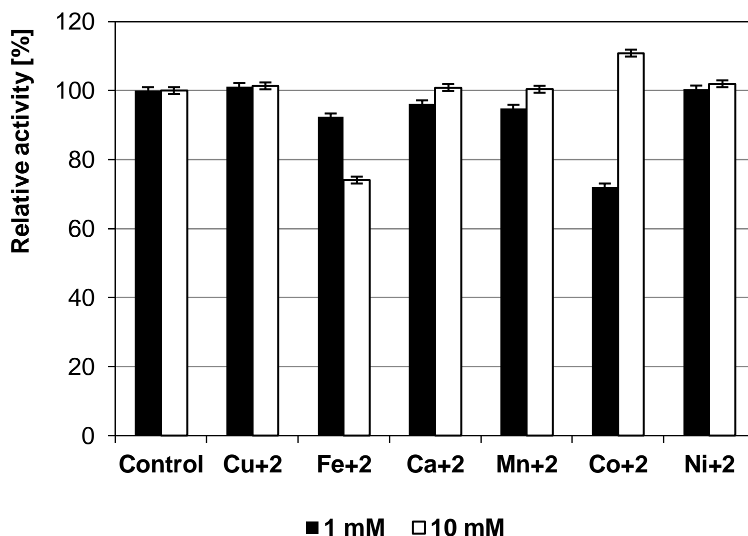

Figure 5. Effects of various metal ions on PPO activity

It has been reported in the literature that Cu^{2+}, Hg^{2+}, Mn^{2+}, Zn^{2+}, Al^{3+}, Ni^{2+}, Cd^{2+}, Fe^{3+}, K^{+}, Ca^{2+} ions at 1 mM concentration have a different effect on the PPO from medlar fruit at different ripening stages (Ayaz et al., 2008). In the study conducted with the borage, it has been reported while some metals (Fe^{+3}, Mg^{2+}, Pb^{2+}, Zn^{2+}, K^{+}, Cu^{2+}) increased PPO activity and some of them (Hg^{+2}, Mn^{2+}, Ni^{2+}, Na^{+}) decreased the activity at 1 mM and 5 mM ion concentrations (Alici and Arabaci, 2016). When the effect of Na^{+}, Hg^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Al^{+3} ions (at the final concentrations of 1 mM and 10 mM) on mushroom PPO activity was examined, the Hg^{2+} ion caused inhibition on PPO activity, by contrast with Na^{+} ve Al^{3+} ions (Kuyumcu, 2014). Gao *et al.* (2009) reported a positive effect on the enzyme activity for K^{+} and Na^{+} ions for the PPO isolated from Swiss chard leaf; while Mg^{2+}, Ca^{2+}, Cu^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+} ions had negative effects. These differences in the effects of metals on the catalytic activity of the enzyme may be due to the difference of metal solutions, the variety of metals and the different valences of metals (monovalent, divalent, trivalent), which makes it difficult to compare the researches.

In order to prolong the shelf life of fruits and vegetables and maintain their quality, various inhibitors are used to prevent enzymatic browning. In this study, the inhibitory effect of L-ascorbic acid, ethylenediamine tetraacetic acid (EDTA), sodium azide, L-cysteine, potassium cyanide and sodium bisulphite was showed on PPO activity (Figure 6). The highest inhibition rates have showed ascorbic acid (78.2±2,1%) and sodium bisulphite (73±2.14%) at a concentration of 6 mM; and sodium azide (48.3±2.1% and 71±1.8%) and L-cysteine (56±2.3% and 69±0.8%) at concentrations of 3 mM and 6 mM. In the study, the IC_{50} value for L-cysteine the most common inhibitor of PPO, was found to be 50 mM. In many previous studies, ascorbic acid, sodium metabisulphite and L-cysteine have been reported to be effective inhibitors on plant-derived PPO enzymes (Paul and Gowda, 2000; Nagai and Suzuki, 2003; Guo et al., 2009).

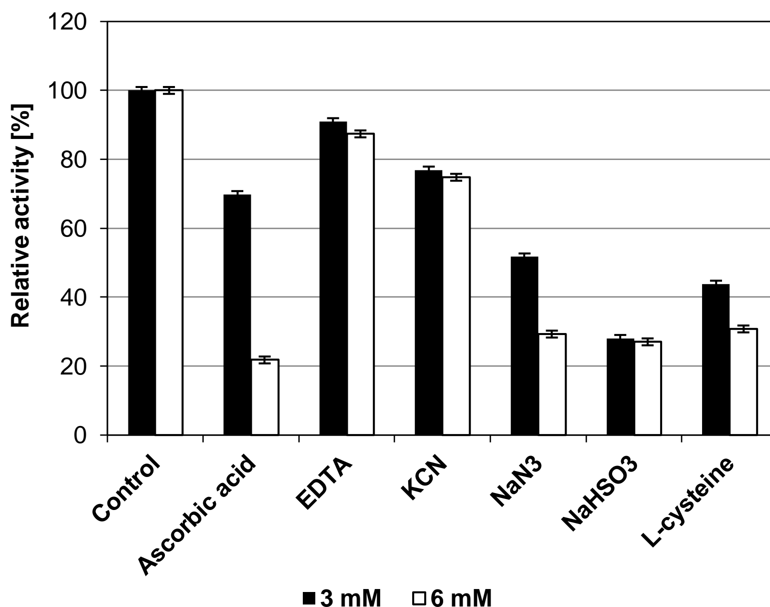

Figure 6. Inhibitor effect of chemicals on PPO activity from faba bean coat

To investigation of the effect of storage stability of enzyme, partially purified PPO from faba bean coat was kept in 0.1 M phosphate buffer (pH 6.0) at +4 ºC over a month period. It was observed that the enzyme solution retained its activity about three weeks, and it lost 67% enzyme activity after 20 days (Figure 7). Our findings are similar to those of other authors. They reported that after storage at +4 °C, activity of PPO decreased about 97% for borage plant after 20 days (Alici and Arabaci, 2016), 90% for blueberry after 3 weeks (Siddiq and Dolan, 2017) and 60% for grape after a month (Onez, 2006), respectively. Nagai and Suzuki (2003), on the other hand, reported that activity of soybean sprouts PPO decreased after 8 days at 4 °C.

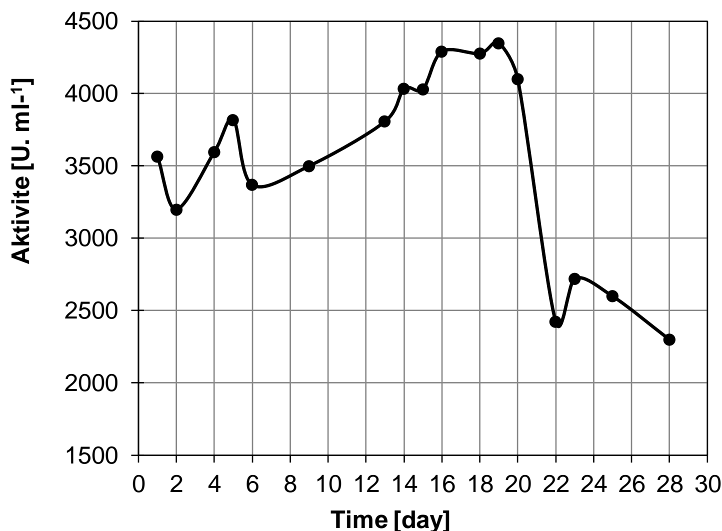

Figure 7. Inhibitor effect of storage time on the activity of faba bean coat PPO at +4 °C

In the literature, few studies have been reported on the isolation and characterization of PPO from faba bean leaf. These works cover investigations of PPO isoforms which have different isoelectric points and molecular weights (Robinson and Dry, 1992; Ganesa et al., 1992; Flurkey, 1989). However, there are studies on PPO from Fabaceae family including green bean (Guo et al., 2009), field bean (Paul and Gowda, 2000), mung bean (Shin et al., 1997) and soy bean sprouts (Nagai and Suzuki, 2003) from the same family with faba bean. On the other hand, Ozcan and Sagiroglu (2014) have designed a biosensor for the determination of phenolic compounds by immobilizing the faba bean shell homogenate to the glassy carbon electrode surface. In their study, various phenolic compounds (catechol, p-cresol, caffeic acid, hydroquinone, pyrogallol, cinnamic acid) were oxidized by the PPO enzyme in the faba bean coat tissue. In the literature, no studies have been conducted on faba bean pod except this amperometric based biosensor study. In future work, it is considered to develop other biosensors in which this plant is used as an enzyme source.

Conclusion

Polyphenol oxidase has notable, since it is the key enzyme responsible for browning and defencing system in the plant kingdom. The researches on PPO can lead to understanding this enzyme that catalyses the browning reaction. Therefore, it is important to understand the biochemical properties of polyphenol oxidase, which is a food quality related enzyme. It was our aim to determine some properties of PPO from faba bean coat such as optimum pH, optimum temperature, substrate specificity, kinetic values, thermal stability, effect of some chemicals and metal ions on enzyme activity. The results demonstrate that the enzyme has heat stability and the possibility of being used to construct biosensors and other analytical methods in various fields. The most effective inhibitors were ascorbic acid and sodium bisulfite, and Fe^{2+} ions caused a decrease in the PPO activity. In conclusion, the enzymatic characterization of the PPO from faba bean coat was defined by us for the first time, and it may help to better understand the PPO enzyme, especially in food industry.

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High pressure applications in low salt gel meat technologies. A review

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Abstract

Introduction. The aim of this work is to analyze and generalize studies on high-pressure effects for gel meat products with low levels of salt (1%), namely, changes in the structure of meat proteins and gel properties.

Materials and methods. Literature analysis.

Results and discussion. A high pressure, as a kind of physical sterilization technology, can effectively change the protein structure and techno-functional features of meat products, while retaining nutritional features and the taste. The effect of water holding capacity, colour, texture and sensory features in low-salt gel meat products under high pressure was reviewed. The effect of processing process parameters (pressure, time and temperature) on low salt gel meat products was analyzed to alter their structure, conformation and gel properties. Meat protein is sensitive to high pressure. The structures of the α-helix and β-sheet changed to those of the random helices and β-turns as the pressure was increasing. The solubility of the protein and the hardness of the gel reach their maximum indicators, and the microstructure of the gel is optimal dense and uniform at 200 MPa. Overall, high pressure processing can be used to produce low-salt gel meat products with good quality. Processing of a beef sausage with a low salt content at a high pressure of 200 MPa leads to increased solubility of myofibrillar proteins such as myosin and actin in salts. The combination of high pressure and heat processing at low salt meat protein denaturation temperatures results in better retention of water and texture than in samples prepared only by heating process. Thus, high pressure processing before heat one improves the functionality of the meat mince.

Conclusions. High-pressure processing can be effectively used in the production of gel meat products with low salt content and high consumer features.

Introduction

Sodium chloride is the main source of human sodium intake, and now the low-salt food has been payed more and more attention in recent years (Pretorius et al., 2018; Pateiro et al., 2021). The world health organization (WHO) recommended that the intake of sodium chloride should be 4-6 g/d, but the average intake of sodium chloride in the most countries was much than that (World Health Organisation, 2007; Zhang et al., 2017; Zhang et al., 2017). Excessive intake of sodium chloride can cause hypertension and cardiovascular diseases, increase the average incidence of kidney diseases and gastric cancer (Bistola et al., 2020; Tobin et al., 2013; Datta et al., 2007). The historical experience of salt reduction shows that reducing the per capita sodium chloride intake requires the effective participation of food processing industry (Aliño et al., 2010; Paula et al., 2019; Li et al., 2021), among which the most effective method is adopt new processing technology to reduce the sodium chloride content in meat products. According to the traditional thermal gel mechanism of meat proteins, sufficient sodium chloride can extract the salt-soluble protein, such as myosin and actin, to form good texture and taste. The way of directly reduced sodium chloride could cause the product yield and edible quality were significantly reduced (Barbut et al., 1988; Desmond et al., 2006; Li et al., 2021). Therefore, how to reduce sodium chloride content while ensuring the good quality of product has become an urgent problem to be solved in the meat industry.

The use of high pressure technology to reduce sodium chloride content in the gel type meat has been reported by some researchers (Barbut et al., 1988; Desmond et al., 2006; Li et al., 2021; Li et al., 2020; Li et al., 2021; Lu et al., 2021; Dixon et al., 2019; Dixon et al., 2019). Due to the structural of protein material component differences appear different degree of compression deformation, when the deformation degree is large enough, it may affect the combination between protein molecules formed, and cause the destruction and restructuring, which affects the functional characteristics of protein. Moreover, protein molecule conformation may bring better functional characteristics due to sudden release of pressure after pressure is withdrawn (Gao et al., 2018; Xu et al., 2019; Wei et al., 2019). The high pressure processing plays a key role in forming the quality of the meat products, there are a few reports on reducing salt content in the meat products, but the mechanism of lowering sodium chloride was still not completely understand (Jayathilakan et al., 2019).

One of prospective directions of improving meat products technologies in the direction of reducing their salt content is a high pressure technology. Its usage does not reduce the amount of introduced sodium chloride from 1.8 to 1.2% in the recipe composition of pork sausage, but also improves its technological and consumerist features (Li et al., 2021). Some general principles and laws that allow developing a goal-oriented strategy for managing meat products technologies describe these changes.

1. Effects of sodium chloride in gel meat products

Sodium chloride plays an important role in gel meat products. First, sodium ions and chloride ions can stimulate taste. Second, myofibrillar protein can be extracted to facilitate the dissolution and swelling, it increases the water- and oil-retaining properties of the gel, and improves product yield, texture and shelf life (Li et al., 2020, Li et al., 2020; Kang et al., 2014; Alvarez et al., 2007; Yao et al., 2017; Inguglia et al., 2017; Kang et al., 2018). The function of dissolving and extracting myofibrillar is called the processing effect of sodium chloride, which is the key to form the quality of gel meat products (Lu et al., 2021; Zhu et

al., 2018; Mancini et al., 2019). The swelling of myofibrillar is also very important for the processing of meat products, it is wrapped around the meat and fat particles or liquid drops. During heating, the substances are cross-linked and the water is trapped in the protein matrix. Sodium ions can form electron clouds around myofibrillar molecules and promote the dissolution of myofibrillar protein (Offer et al., 1983). In fact, the sodium chloride content from 1 to 1.5% can meet the majority of consumers' demand for salty taste, however, to meet the processing required, the gel meat products generally add 2 to 4% sodium chloride (Hand et al., 1982).

The biggest obstacle to reduce sodium chloride in meat products is that sodium chloride is a very cheap ingredient, and consumers are more comfortable with the quality and flavor of meat products with adding salt. On the premise that consumers can accept, the main ways to reduce the sodium content of gel meat products are summarized as follows: reduced the amount of sodium chloride added and replaced with other salt; the Glutamine transaminase is added to catalyze the interprotein (or internal) acyl transfer reaction to form covalent cross-linking between proteins (or polypeptides) (Colmenero et al., 2005; Kang et al., 2016). The new process can improve the performance of myofibrillar, and still form a good thermally induced gel at low ionic strength (Desmond et al., 2006; Inguglia et al., 2017; Kang et al., 2016).

The overall use of salt substitutes is difficult for consumers to accept, but the partial reduction of salt in meat products is a desirable approach. There are many kinds of salt, but few of them can be used to process gel meat products successfully or completely instead of salt (Inguglia et al., 2017; Kang et al., 2017; Mariutti et al., 2017).

In most studies, sodium chloride was replaced by other chloride salts, because the processing effect of sodium chloride is mainly achieved through the binding of chloride ions with proteins. The decrease of chloride ion content is lead to a significant decline in gel properties. At present, the most successful alternative salt in the study is potassium chloride, which can replace about 35~40% sodium chloride in the formula of gel meat products, but excessive potassium chloride will produce bitter and other bad smells (Zhang et al., 2017). Polyphosphate can increase the pH of meat chyme, cause muscle fibrils to swell, facilitate actomyosin dissociation, so it can partially replace sodium chloride. Reducing sodium chloride crystal size and changing crystal shape can reduce the amount of sodium chloride added without affecting food saltiness. However, gel meat products contain a lot of water, and sodium chloride is dissolved in water, so the effect of reducing the content of sodium chloride in gel meat products, thus, this method is limited (Angus et al., 2006).

Another way is to use flavor enhancers. Flavor enhancers increase the saltiness and flavor of low-salt meat products, it decrease the use of salt without reducing the saltiness and flavor of meat products. Some flavor enhancers and shaders have been used in industrial production, and the usage is increasing, such the products include yeast extract, lactate, sodium glutamate and nucleotide. Flavor enhancers can stimulate the taste and reduce the stimulation of sodium chloride to the taste nerve, helping to reduce the amount of sodium chloride used. Pasin et al. (1989) used potassium chloride and nucleotide mixture (50% IMP and GMP mixture used commercially) to reduce salt in pork sausages by 75%; Any amount of glutamate in these pork sausages combined with potassium chloride can be substituted for 50% salt (Pasin et al., 1989). Ruusunen (Ruusunen et al., 2001) found that adding glutamate sodium or nucleotide mixture to bonian-type sausages could enhance their flavor, there was not significant changed after 17 days stored, and the sausage with adding glutamate sodium or nucleotide mixture had a better overall acceptability, while the sausage with adding flavour enhancer had a good edible quality (Ruusunen et al., 2001). Other compound flavor enhancers, such as lysine and succinic acid mixtures, have been studied as substitutes for salt.

This kind of complex has the flavor of salt, as well as antibacterial and antioxidant properties. It can replace 75% of salt and has a good development prospect (Triki et al., 2017). Through adding phosphate, starch and hydrocolloid can make up for the effect of decreased water retention and product quality of low-salt meat products. The use of different levels of potassium lactate or sodium lactate as an alternative salt to maintain the product's flavor and saltiness (Omana et al., 2011).

Due to the differences in muscle physiology and structure, especially the composition of free amino acids that can react with glutamine transaminase, such as the number and distribution of amino acid residues, the effects of different types of muscle proteins are different, but the heavy chain of myosin is sensitive to glutamine transaminase. Transglutaminase greatly changes the structure of myosin heavy chain, leads to the lignin-helical structure is reduced, and the lignin-folding structure is increased, resulting in the generation of polymers, improving the texture of the gel and forming a orderly gel structure (Luisa et al., 2015). The other, added glutamine transaminase into 1% sodium chloride content of chicken meatballs can improve the yield and gel strength, forming a dense and ordered gel structure (Fellendorf et al., 2016). Edrosolam et al. found that added glutamine transaminase into low-salt and low-fat bonija sausage, who found that the addition amount of 0.15% could increase the elasticity, hardness and cohesion of the hot gel (Edrosolam et al., 2014).

2. The principle of high pressure processing

High pressure processing (HPP), can be referred to as ultra high pressure technology or hydrostatic technology, the water or other incompressible fluid mediums often act as mediators of pressure. During the high pressure processing, the pressure levels generally not less than 100 MPa, the commonly used range is 100–1000 MPa and can work in the temperature range of -20 to 90 °C. After the food is sealed in an elastic container or placed in a pressure system, the non-covalent bonds (hydrogen bonds, ionic bonds and hydrophobic bonds, etc.) are been destroyed or formed at a certain temperature for the appropriate processing time and pressure level, which cited the enzyme in food, protein, starch and other biological high molecular substances are deactivated, denatured and gelatinized respectively, and kill the microorganism in food biological, so as to achieve the purpose of food sterilization, preservation and processing (Wael et al., 2015).

As with heat, pressure is a basic thermodynamic variable. Strictly speaking, during HPP the effects of temperature cannot be separated from the effects of pressure. This is because for every temperature there is a corresponding pressure. Thermal effects during pressure treatment can cause volume and energy changes. However, pressure primarily affects the volume of the product being processed. The combined net effect duringHPPmay be synergistic, antagonistic, or additive (Koutchma T. et al., 2012).

Mathematically, the impact of pressure (p) and temperature (T) can be quantitatively related using Gibbs's definition of free energy G (Koutchma T. et al., 2012):

$$G \equiv H - TS, \tag{1}$$

where H and S are the enthalpy and entropy, respectively. Further,

$$H \equiv U + pV, \tag{2}$$

where U = internal energy and V = volume.

It can be deduced from Equations 1 and 2 that

$$d\,(\Delta G) = \Delta V dp - \Delta S dT\,. \quad (3)$$

Therefore, reactions such as phase transitions or molecular reorientation depend on both temperature and pressure and cannot be treated separately.Thefollowing are some basic governing principles behind HPP.

The fundamental principles of hyperbaric technique are Pascal's law and Le Chatelier principle. Pascal's law takes advantage of the compression effect of high pressure on liquids, which means that the pressure applied to the liquid can be transmitted to all parts of the system instantaneously at the same size. Therefore, dry food, powdery food or granular food should not be used high pressure treatment. According to Pascaline law, the effect of high pressure processing is independent of the size, shape and volume of the food. In the process of high pressure processing, the whole food will be treated uniformly, the pressure transfer speed is fast, there is no pressure gradient.Therefore, the high pressure processing of food is simpler, and the energy consumption is also significantly reduced. According to Le Chatelier principle, the external pressure reduces the volume of the pressurized system and vice versa. Therefore, the physical and chemical reactions in food ingredients will be carried out in the direction of the maximum compression state under the pressure treatment of food. The increase or decrease of the reaction rate constant k depends on whether the "active volume" of the reaction is positive or negative. This means that high pressure processed food will force the reaction system to reduce the volume, affecting not only the reaction balance in the food, but also the reaction rate, including chemical reactions and possible changes in molecular conformation. It is well known that the mechanism of meat proteins unfolded, denaturation and formed gel caused by heat and high pressure is difference. High pressure processing induced meat gels are based on the protein volume decline, while the thermal meat gels is caused by the violent movement of molecules and destruction of non-covalent bonds.

At constant temperature, an increase in pressure increases the degree of ordering of molecules of a given substance. Therefore, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions.

As with thermal processing, various reaction rates during HPP are also influenced by thermal effects during pressure treatment. The net pressure-thermal effects can be synergistic, additive, or antagonistic.

High-pressure processing of muscle based products is paying more and more attention in the meat industry, which could prolong the shelf life of meat products, inactivate vegetative micro-organisms and enzymes near room temperature, because of the processing allows the decontamination of muscle based products with minimal impact on their nutritional and sensory features. Therefore, The application of high pressure offers some interesting opportunities in the processing of muscle-based food products, such as, the high pressure can affect the texture and gel-forming properties of meat batter and myofibrillar proteins, the tenderize, color and other properties of muscle. The processing effects on muscle based products are highly dependent on the primary effects of pressure, time and temperature on the relevant thermodynamic and transport properties of meat systems. However, the pressure-labile nature of some meat protein systems, such as myosin or myoglobin often limits the range of attractive commercial applications to prefermented and cooked meat products.

3. Use of high pressure in meat products

Improving processing techniques and equipment can also reduce sodium chloride of meat products. The application of high pressure technology in food has a long history. As early as 1895, Royer found that high pressure treatment can kill bacteria. In 1899, Hite first discovered that 450 MPa pressure could extend the storage period of cow milk. Brigman, an American physicist with the reputation of "pioneering the study of modern high pressure technology", who has been conducting systematic research on the high pressure effects of macroscopic behaviors such as solid compressibility, mechanical properties. In 1914, the high pressure technology of food was defined, and report that the albumin solidified under 500 MPa and became hard gel under 700 MPa was proposed (Bridgman et al., 1914). High pressure treatment led to change in the structure of protein molecules by affecting the molecular volume and non-covalent bond of protein, thus improving their functional activity (Ngarize et al., 2004; Xu et al., 2016).

3.1. Effect of high pressure on tenderness in meat products

High pressure treatment has a certain tenderizing effect on meat. The tenderness of meat after high pressure treatment is increased, indicates that the muscle node structure is damaged and the muscle fiber structure is changed during pressure treatment (Tauc. et al., 2002; Perrett et al., 2002; . Ma et al., 2013; Warner et al., 2019). The tenderizing mechanism of high pressure on meat mainly has two aspects:

1. Mechanical force causes the binding dissociation of muscle actin and myosin in muscle fibers, muscle fiber disintegrates and muscle fibrin dissociates into small fragments, resulting in a decrease in muscle shear force;
2. Pressure treatment causes the activation of endogenous proteinase-calcium enzymes in muscles (Cioni et al., 2002; Simonin et al., 2012).

Accompanied by the tenderizing, the changes of meat color was generated treated by high pressure. The myoglobin and hemoglobin in the myoplasm gradually denature under pressure and lose their inherent red color, resulting in the red color of muscles gradually becoming lighter and gray, and finally turning gray like cooking meat (Orlien et al., 2014; Wang et al., 2020). When the pressure is lower than 200 MPa, the appearance of meat does not change much, but the color of meat fades when the pressure is higher than 200 MPa. The reasons of pressure processing of meat color are as follows:

1. The pressure between 200~350 MPa, the white color (L* value) was probably globulin of myoglobin degeneration or heme is replaced or lose.
2. The pressure loss of red when more than 400 MPa, due to ferrous myoglobin oxidation into high-speed rail by myoglobin (Canto et al., 2012; Ha, 2017). The occurrence of the above two changes are main dependent on whether exceeds the pressure required for the change, and less affected by the pressure and time.

3.2. Effect of high pressure on oxidation in meat products

Some studies have investigated that the influence of high pressure on fat stability of meat products is related to oxygen, composition of meat and temperature. The effect of high pressure on lipids is reversible, and lipids are stable under appropriate high pressure. In the presence of meat tissue, the pressure at room temperature over 300 MPa is aggravated the rate of lipid oxidation, and the TBA value of peroxides is higher than that of unpressurized lipids (Tuboly et al., 2003; Chen et al., 2018; Bernasconi et al., 2020; Rivas-Caedo et al.,

2021; Martínez-Onandi et al., 2019). Under the condition of high temperature, the inhibition effect of high pressure treatment on fat oxidation will disappear. High pressure induced fat oxidation limits the application in meat processing. Metal chelating mixture can effectively eliminate the initiation of fat oxidation and become an ideal antioxidant to prevent fat oxidation in meat treated by high pressure (Guyona et al., 2016; Cava et al., 2020).

3.3. Effect of high pressure on bacteria in meat products

High pressure can destroy the cell wall and cell membrane of bacteria, inhibit the activity of enzymes and the replication of genetic materials such as DNA, destroy the hydrogen bond, disulphide bond and ionic bond of proteins, and finally cause the death of microorganisms (Delgado et al., 2015; Park et al., 2006; Garriga et al., 2002). As the temperature increases (decreases) with the pressure (pressure relief) during the high pressure treatment, the temperature increases 2~4 °C for every 100 MPa of pressure increase. Thus, in recent years, it has also been considered that the lethal effect on microorganisms is the result of the combined action of compression heat and high pressure (Cava et al., 2020; Yuste et al., 1999; Ko et al., 2002; Wang et al., 2021). It is well known that gram-negative bacteria and yeast can be basically killed at a pressure of about 400 MPa, while gram-positive bacteria need to be killed at a pressure of 600 MPa, while to kill spore bacteria requires a higher pressure and proper heating and prolongs holding time. The killing effect of spore bacteria can be improved obviously if the high pressure pulse treatment is applied alternately to pressure and pressure relief (Bonilauri et al., 2021; Jung et al., 2003; Macfarlane et al., 1985).

4. The use of high pressure in low-salt meat products

The high pressure treatment improves the functional properties of meat protein, and is beneficial for reducing salt (Chen et al., 2018; Duranton. et al., 2012; Zheng et al., 2017). Such as, the surface hydrophobicity and total sulfhydryl groups of rabbit myosin were increased under high pressure between 100 and 200 MPa (Chapleau et al., 2004). Meat protein is sensitive to the high pressure treatment. The α-helix and β-sheet structures changed into random coil and β-turn structures as the pressure levels increased; moreover, the protein solubility and gel hardness reached their maximum values and the gel microstructure was dense and uniform at 200 MPa. Thus, a better understanding of the changes in gel properties and protein conformations occurring in meat products induced by combined high pressure and thermal conditions could be helpful to elucidate their role during gel formation and facilitate the development of new healthy meat products (Zheng et al., 2017; Zhang et al., 2017; Yang H et al., 2021).

Sensory evaluation was conducted after the high pressure treatment of low-salt frankfurter by high pressure, and it was found that the tasters were more likely to accept the sausages treated with low salt and high pressure, which indicated that high pressure could improve the texture of the sausages, and partially reduce the amount of salt (Crehan et al., 2000). Grossi et al. (Grossi et al., 2011) reported that the use of high pressure technology could reduce the sodium chloride content from 1.8% to 1.2% of pork sausage with carrot fiber and potato starch, and had no negative impact on the water-retaining performance, color and texture. The other, increased the pressure and temperature, and the meat batter with carrot fiber forms a highly elastic, organically combined and orderly network structure (Grossi et al., 2011; Sun J., et al., 2012).

4.1. Effect of high pressure on cooking yield in low-salt meat products

The water and fat holding capacity is an important indicator the quality of meat products, it expresses the ability of comminuted meat products to hold water and fat. The pressure intensity, salt content, meat type, composition, temperature and others factors independently affected both the water and fat holding capacity of comminuted meat products (Sukmanov et al., 2019a; Sukmanov et al., 2019b). Zheng et al. (Zheng et al., 2017) reported that the cooking loss of low-salt chicken meat batters treat by high pressure was not decreased, this suggested that high pressure was much more effective, than salt in reducing water loss during the cooking. Because of the 100 or 200 MPa is too low to affect the exposure of buried sulfhydryl groups, the SH content of myosin was not significantly differences; at 300 MPa and above, the SH content was significantly increased, the increase of sulfhydryl groups might be explained by the change of myosin structure. Rospolski et al. (Rospolski et al., 2015) studied the effects of high pressure parameters and NaCl concentration on the physical properties chicken meat batter, showed that the water became slightly more tightly bound to the meat matrix after high pressure processing treatment, the reason is that high pressure processing could increase the solubility of meat protein, so that, increased water and fat holding capacity and decreased mechanical water loss (Hugas et al., 2002; Chan J et al., 2011).

4.2. Effect of high pressure on texture in low-salt meat products

The texture is an important characteristic of meat products, which decides the edible quality of low-salt meat product. It is well known that high pressure processing is an important thermodynamic parameter that can profoundly influence molecular systems. Due to the myofibrillar protein became unfolded with the pressure increased, more buried hydrophobic residues were exposure and more hydrophobic sites or pockets of protein molecules, then the large protein aggregates was formed. When the pressures over 400 MPa can readily denature proteins, and 200 MPa only affects their quaternary structures, leading to the dissociation of oligomeric proteins (Aertsen 2009; Bai et al., 2021). Ngarize et al. (2004), Grossi et al., (2016) found that the solubility of myofibrillar proteins was decreased when the pressure up to 400 MPa and above, due to the protein-protein interaction at 400 MPa is formed at the expense of protein-water interactions, and the intermolecular H-bonds between proteins are stronger than the H-bonds between protein and water. Yang et al. (Yang et al., 2015) have investigated that the use of high pressure processing for enhancing the functional properties of reduced-fat (20%) and reduced-salt (1%) pork sausages without the need for additives, who found that the textural properties of hardness, chewiness, springiness, cohesiveness and resilience were significantly ($P < 0.05$) increased at an interval of 100 MPa and 200 MPa, except the adhesiveness up to 200 MPa, but no changes of of hardness, chewiness, springiness and resilience were observed up to 300 MPa and 400 MPa. Because the myofibrillar proteins, such as myosin and actin, were more salt soluble when low-salt beef sausage batters were subjected to high pressure at 200 MPa than the untreated batter (Sikes et al., 2009; Tintchev et al., 2013). (Oflynn et al., 2014) found that high pressure treatment is a potential technology to manufacture sausages maintaining sensory and functional properties, and could decrease the salt levels in reduced-phosphate breakfast sausages, improve the juiciness and cohesiveness.

4.3. Effect of high pressure and heat combination in low-salt meat products

It is well known that the mechanism of meat protein denaturation and formed gel caused by heat and high pressure are differences. The high pressure treatment induced meat gels is based on the protein volume decline, while the thermal meat gels is caused by the violent movement of molecules and destruction of non-covalent bonds. Some researches have reported the effected of low-salt meat products on the combination of heat and high pressure (Chen et al., 2018; Zheng et al., 2017; Tintchev et al., 2013; Oflynn et al., 2014; Khan. et al., 2014; Zheng H. et al., 2019). The temperature during high pressure processing also affected water and fat holding capacity, and gel properties. Combining high pressure and heat treatment at low-salt meat protein denaturation temperatures in a single-step process has reportedly resulted in better water retention and texture than heat-only samples (Jimenez-Colmenero et al., 1998; Zheng et al., 2017; Zheng. et al., 2015). High pressure treatment prior to thermal processing improves the functionality of meat batters. Wei et al. (Wei et al., 2019) studied the effects of protein conformations and gel characteristics of low-salt (1% sodium chloride) pork batters produced by high pressure prior to heating (20-60 °C), who found that the highest cooking yield, hardness, springiness, chewiness, and G' values were observed in batters made by high pressure at 20 and 30 °C. Meanwhile, the α-helix structure was significantly decreased, and accompanied by the increase of β-sheet, β-turn, and random coil structures at 20-40 °C. The reason is possible that the maximal solubilization of myofibrillar protein occurred at 200 MPa, with a reduction of salt content by 50% and improvement of functional properties, such as water-holding capacity and texture (Tintchev et al., 2013). Zheng et al. (Zheng H. et al., 2019) investigated the effect of appearance, texture, water holding capacity, sensory attributes and microstructure of chicken breast meat batters by heating under 0–400 MPa (75 °C, 30 min), the result was that the high pressure, rather than salt, was the main factor affecting the quality of chicken meat batter, the quality of low-salt chicken batter was improved by heating under 200 MPa and formed a fibrous network inside muscle fibers; meanwhile, application of HUP at a specified pressure was an excellent process for producing low-salt comminuted meat products; but excessive high-pressure resulted in inferior quality.

Conclusion

High pressure has been widely applied and developed rapidly in practical production due to its leading technical characteristics, good economic and social benefits. The effects of high pressure on various components of low-salt meat products vary greatly with the pressure, temperature and time. In spite of great efforts, the mechanism of high pressure on low-salt meat products has not yet been obtained, which leads to get a clear understanding of their behaviour is difficult. Even so, with the development of research, people will have a better understanding on the influence of high pressure in low-salt meat products and its relationship with the change of meat quality. Thus, the use of high pressure processing could improve the quality of low-salt meat products.

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Evaluation of steaming time on the colour and physical properties of four paddy rice varieties in West Africa

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Abstract

Introduction. Steaming in rice-parboiling causes gelatinization and protein disintegration in the endosperm changing its appearance and strength by fused gelatinized starch granules that disrupt protein bodies.

Materials and methods. Steaming-periods on grain strength and appearance of FARO64, FARO65, FARO66 and FARO67 rice varieties were evaluated. Each variety was divided into 5-parts; 4-parts parboiled and steamed for 5, 10, 15 and 20 mins (at constant temperature and atmospheric pressure), while the 5th portion served as control.

Results and discussion. Steam application had a significant effect ($p<0.05$) on grain strength. Optimum strength was recorded at 20 mins steaming-period in FARO64, FARO65 and FARO66, while FARO67 had highest strength at 15 mins with values ranging from 61.53N to 225.83N. FARO65 had reduced L* value, though all varieties kept the yellow colour with the steaming-time. Only FARO64 maintained a* values with steaming-time. For length, FARO67 changed to very long, while FARO64, FARO65 and FARO66 changed from medium to long with a value range of between 6.33 mm and 7.57 mm. The width values (1.98-2.62 mm) equally changed, as all varieties increased with steaming-time. All except FARO64 reduced in thickness with steaming-time. Shape of FARO64 changed from medium to slender; FARO65 and FARO66 maintained medium shape, while FARO67, from slender to medium. Thousand grain weights were between 15 and 24; FARO64, FARO66 and FARO67 had reduced mean-value, while FARO65 increased.

Conclusion. Parboiling reduces raw rice breakage rate, and improves strength, colour and appearance. Gelatinization temperature influences rice quality.

Introduction

Rice is a staple food for about half of the world's population (Ebuehi *et al*., 2007); ranked as number one cereal; cooked and consumed in milled form (Hossain *et al*., 2009). Processing operations, pre-harvest to postharvest, affect milled rice quality, as over 90% of Nigerian rice is parboiled before consumption. Parboiling operations involves soaking, steaming and drying, as applied to rough rice for improved quality attributes, changes in milling properties, increase strength, while the protein denaturation diffuses into inter-granular space of starch, which further increases the binding effect for better milling process (Islam *et al.,* 2004; Elbert *et al*., 2000). Steaming gelatinizes and concentrates nutrients on husk and bran layers, which then migrates inward through the solute via retrogradation process. Steamed rice becomes compact, thus making milling process easier since the husk's kernel is loose (Ayamdoo *et al.,* 2013; Vikrant *et al*., 2018). Starch of the gelatinized and parboiled rice usually experience changes in appearance (Singh *et al*., 2000), eliminating the usual opaque portion in rice and making the end product more translucent and glossier than the un-parboiled rice.

Worldwide, rice is usually consumed as whole grain, where its physical properties such as colour, dimension and appearance determine quality, which are initial requirements for new rice variety in accordance to its size and shape, appearance, weight and uniformity (Varnamkhasti *et al.,* 2008; Danbaba *et al*., 2012). Rice dimension, to a large extent, helps in equipment design for shape classification, selection of membrane size for optimal separation and in calculating machine milling efficiency (Singh *et al*., 2015). Rice length determines the breakage level, but steaming process better strengthened rice grain during parboiling, and reduced breakage (Kumar, 2013). Rice shape is usually determined via length and width ratio; however, its thickness (breath) reflects nutrient density, which invariably, amount to the physicochemical properties of the rice (Bocevska *et al*., 2009). For these parameters, rice is classified based on their length into very long (>7.5mm), long (<6.61mm), medium (5.51-6.1mm) and short (<5.5mm) and shapes with the use of length to width ratio (IRRI, 2009).

Rice users, either in the cooked or raw form, constantly emphasize particularly on the appropriate quality of rice. However, rice varieties vary in quality and properties with respect to plant and grain characteristics, plant type, height, nature of leaves, grain dimension, texture, starch content, colour, aroma, cooking properties and nutritive quality (Amaka *et al*., 2014), though each variety varies with specific optimum thermal treatment appropriate for its parboiling processes for high quality product (Igathinathane *et al*., 2005). Studying effect of steam application on the selected new indigenous rice varieties is very important for optimum quality and consumer acceptability, as adequate information on the new varieties is important to know whether they are comparable to those already in the market. Rice farmers, millers and consumers need adequate information on the quality of the indigenous new rice varieties. Based on these facts, this study aimed at evaluating the qualities of some new parboiled rice varieties steamed at various time-periods (using constant temperature and atmospheric pressure) in comparison with un-parboiled milled samples.

Materials and methods

Sourcing of materials

Four new rice varieties, FARO64, FARO65, FARO66 and FARO67 were obtained from the farms of National Cereals Research Institute, Badeggi, Niger State, Nigeria and processed according to the method of Danbaba *et al.* (2016) with slight modification in the use of different

steaming periods (5, 10, 15 and 20 minutes) at 100^0C and atmospheric pressure, while the control sample was not parboiled.

Sample preparation

Rough rice was cleaned to remove dockages, and divided into five portions; four portions were packaged in a parboiling bag, soaked in hot water at 65^0C for a period of 3 hours. It was then allowed to stand for 8hrs for conditioning before being steamed at 100^0C (at atmospheric pressure) using an improved equipment (Water bath, TT-420.Techmel and Techmel, USA) that ensures uniform steam distribution between 5 and 20mins. Drying of the steamed samples was done at ambient temperature (25±2^0C) and atmospheric pressure to reduce moisture to between 13-14%, and later milled using a laboratory model rubber roll rice huller (THU35B Satake ENGINEERING CORP, Tokyo, Japan) to de-husk into brown rice, which was polished in Rice-pal 32 (Yamamoto Co, Higashine, Japan) for a period of 3mins (at Food Technology and Value Addition Programme Laboratory), National Cereals Research Institute, Badeggi, Niger State of Nigeria.

Colour measurement

Colour parameters (L^*, a^*, b^*) of milled rice grain was determined with a colour meter (Konica Minolta, CM-3500d, Minolta Co., Ltd. Osaka, Japan), calibrated with a white standard plate (13371004), with illuminant D65 (CIE 196410° Standard Observer) used. The colour coordinates, L^*, a^* and b^* were calculated in the CIELAB system. The L^* values indicates lightness (100 = white and 0 = black); a^*, the degree of red-green and b^*, level of yellow-blue colour, with higher b^* indicating yellowness; replicate readings of 10 to eliminate variations.

Strength of the rice samples

The method of Sunday *et al.* (2018) was adopted for rice strength using a material testing machine (H50 K-S, Hounsfield, England). Briefly, a grain of rice sample was placed on a flat plate of the Instron testing machine, with tester handle turning continuously until 12mm diameter probe pressed the grain with a 500N load cell fixed parallel to the base at a cross-head speed of 1mm/min, which cracks the grain under pressure. Applied force at initial break (rupture) was recorded from the digital computer as the yield point for each run. Five replicates were recorded for each sample.

Measurement of grain dimension

The grain dimension was measured with the method of the International Rice Research Institute (IRRI, 2009). Length, width and breath of randomly selected ten whole grains of milled rice was measured using digital Venire Caliper and grain shape determined through ratio of length to width of grains.

Thousand grain weight

The method of Varnamkhasti *et al.*, 2008 was adopted for weight of a thousand grains, with random selection from bulk of rice grains, and weighed with sensitive weighing balance.

Statistical analysis

Data from 20 rice samples were subjected to analysis of variance (ANOVA) using statistical package for social statistics (SPSS version 20.0); significant differences were compared with least significant difference (LSD) at 5% level of probability, with chat on excel version 2010 (Microsoft cooperation, USA).

Results and discussion

Colour of steamed milled rice

Colour of rice grain is an important attribute of parboiled rice, which usually defines its quality before end users, as well as its market value. Normally, parboiled rice is amber in appearance, which could be darker depending on its physicochemical properties and applied heat during processing. Judging by this, it could be said that quality of parboiled rice is related to steaming time (Ebrahim *et al.*, 2015), and from Table 1, whiteness (L^* value) of FARO64 steamed for 5-20mins during parboiling process was not significant ($p<0.05$) when compared with un-parboiled sample. The L^* values ranged from 55.54 to 56.76 with steaming-time, and different from the reports of Islam *et al.* (2002); Singh *et al.* (2011); Chijoke *et al.* (2013) and Ebrahim *et al.* (2015), where they stated that temperature, soaking period, and steaming negatively influence whiteness of parboiled rice, but Amaka *et al.* (2014) had a contrary view. FARO64 a^* parameters were greenish in colour and significantly different ($p<0.05$). Sample steamed for 5mins, though had the highest a^* value (-3.70), but not significantly different from samples steamed for 10 and 15 mins, and lesser than that of Amaka *et al.* (2014), that reported -1.01 to 1.52. For the b^* values, sample steamed for 20mins (31.92) was not significantly different from the other steamed samples but different significantly from the un-parboiled sample (28.90). The values increased with steaming-time (30.08, 30.76, 31.18 and 31.92) from 5 to 20mins respectively and equally higher than that of FARO61 (23.82) and FARO60 (18.15) as reported by Mayowa *et al.* (2017).

L^* values of FARO65 samples exhibited erratic behaviours. The control sample was not significantly different from sample steamed for 10mins, while samples steamed for 5, 15 and 20mins respectively showed no significant difference ($p<0.05$) among themselves. The applied heat and genetic make-up of the variety could have caused the colour change, or could be attributed it to non-enzymatic browning reaction. The values obtained were in agreement with that of Prem *et al.* (2019) that worked on the effect of steaming on accelerated aging, and Sareepuang *et al.* (2008) that reported on the effect of steam temperature and time on rice parboiling. FARO65 showed varying colour for both un-parboiled and parboiled samples, with un-parboiled having a value of -6.84 and greenish colour. However, parboiled samples steamed at different time-periods were reddish in colour, though redness reduced in value with increased steaming (10mins to 20 mins) from 1.04 in sample steamed for 10mins to 0.30 at 20mins,and significantly different ($P<0.05$) from one another. Ebrahim *et al.* (2015) however stated differently that increasing steaming-time during rice parboiling caused increase in a^* value. The degree of yellowness among FARO65 samples ranged from 28.90 to 27.66 for samples steamed for 10 and 20mins respectively. The b^* value increased from 28.10 in un-parboiled sample to 28.30 and 28.90 in samples steamed for 5 and 10mins, and then decreased in samples steamed for 15 and 20mins, contrary to the report of Ebrahim *et al.* (2015), who reported complete increase in b^* value (18.08 to 19.50). The reduction in the value at 15 and 20mins steaming periods could be due to the intense/prolonged heating period.

Regarding FARO66, the un-parboiled sample recorded highest *L** value (54.68), though not significantly different from sample steamed for 20mins. According to Islam *et al.*, 2008, parboiling tends to darken or reduce lightness colour of parboiled milled rice. Samples steamed for 5 to 15mins were not significantly different ($p<0.05$) from one another, but were significant to that steamed for 20mins, i.e. getting lighter with increasing steaming periods. This revealed that steaming application tends to increase its lightness value. For *a** values, treated FARO66 samples were significantly different ($p<0.05$) from the untreated one (low value of -5.70) with greenish colour. Samples steamed for 5, 10 and 20mins respectively were not different significantly from one another, but were significantly different from the sample steamed for 15mins. Colour change noticed could be due to parboiling effect as earlier stated, which may be maillard type of non-enzymatic browning enhanced by the level of reducing sugar and amino acids during steaming, processing conditions during soaking and steaming, as well as husk pigment that diffused into the endosperm during soaking (Islam *et al.,* 2002). During steaming, nutrients migrated into the endosperm from the husk and bran layer (Bloussi *et al.*, 2010). The *b** values ranged from 27.90 to 29.08. The result showed that the controlled sample (untreated) and samples steamed for 10 and 15mins were not significantly different from one another, while sample steamed for 5mins was different from all the other samples, and comparable to that reported by Graham *et al.* (2015). It was noted that one of the parboiled rice samples had lowest *L** value (dark) and the highest *b** values (yellow) when treated and untreated samples were compared, and this could be attributable to soaking temperature and steaming-time or the effect of steaming on the genetic/agronomic properties of the paddy rice.

Significant difference ($p<0.05$) was observed in the *L** values among FARO67 samples (steamed and un-steamed). Sample steamed for 20mins had the lowest value (52.44), while the highest value (54.80) was recorded with 15mins steaming. The low value of samples steamed for 20mins may be due to the high steam heat that might have darkened the colour of the milled rice (Graham *et al.,* 2015). Sample steamed for 5 and 15mins, as well as the control sample were not significantly different from one another, likewise samples steamed for 10 and 20mins. Lightness value decreased with increased steaming-time, except in the 15mins steaming. The *a** values of FARO67 were significant. Un-parboiled sample was significantly different ($p<0.05$) from the treated samples. Colour of parboiled rice changed gradually from greenish to reddish with increase in steaming-time, but changed after 15mins. Un-parboiled sample of FARO67 was greenish (-6.10) in colour, but samples steamed for 10 and 15mins were reddish and similar, while samples steamed for 20 and 5mins respectively were greenish in colour (-0.06 and -022). It could be concluded that longer steaming period in rice parboiling had negative effect on *a** value of processed rice, which could be attributable to genetic make-up according to Islam *et al*., 2002. FARO67 samples were not necessarily significant for *b** values. According to Lamberts *et al.,* 2008, high heat treatment during parboiling caused reduction in carotenoids of raw rice and maillard reaction and imparting yellow colour to the samples. It could be said generally that steaming had great effect on the colour attributes (either through the genetic make-up or agronomic properties) of the rice varieties.

Table 1

Effect of Steaming-Time on the Colour of parboiled rice

Sample	Rice variety	Steaming period	L*	a*	b*
64	FARO64	Non parboiled	**55.54±0.09[a]**	**-6.46±0.05[f]**	**28.90±0.07[bc]**
564		5	55.36±0.01[a]	-3.70±0.02[e]	30.08±0.04[ab]
1064		10	56.34±0.1[a]	-3.98±0.02[e]	30.76±0.02[ab]
1564		15	56.66±0.1[a]	-3.92±0.06[e]	31.18±0.14[a]
2064		20	56.76±0.15[a]	-4.56±0.03[f]	31.92±0.07[a]
65	FARO65	Non parboiled	**54.70±0.15[b]**	**-6.84±0.06[f]**	**28.10±0.05[bc]**
565		5	52.12±0.09[c]	0.92±0.07[a]	28.30±0.07[bc]
1065		10	54.32±0.21[b]	1.04±0.03[a]	28.90±0.10[bc]
1565		15	51.48±0.19[C]	0.60±0.02[b]	27.28±0.09[c]
2065		20	51.70±0.22[C]	0.30±0.03[c]	27.66±0.14[c]
66	FARO66	Non parboiled	**54.68±0.05[b]**	**-5.70±0.02[f]**	**28.62±0.01[bc]**
566		5	51.70±0.29[C]	0.84±0.06[a]	27.90±0.11[c]
1066		10	51.16±0.29[C]	0.88±0.05[a]	28.58±0.18[bc]
1566		15	52.20±0.20[c]	0.64±0.05[b]	28.36±0.0[bc]
2066		20	54.32±0.35[b]	0.86±0.05[a]	29.08±0.17[b]
67	FARO67	Non parboiled	**53.92±0.05[b]**	**-6.10±0.09[f]**	**28.16±0.03[bc]**
567		5	53.88±0.11[b]	-0.22±0.08[d]	29.24±0.04[b]
1067		10	52.90±0.21[c]	0.40±0.04[b]	28.58±0.08[bc]
1567		15	54.80±0.33[b]	0.40±0.02[b]	29.14±0.09[b]
2067		20	52.44±0.24[c]	-0.06±0.03[cd]	28.12±0.14[bc]

Effect of Steaming-Time on the Strength of Parboiled Milled Rice Samples

Steaming operation in rice parboiling improves the quality of rough rice by sealing cracks in rice kernel (hardened) to resist milling forces, thus reducing rice breakage during milling. It gives high milling recovery, better market value, higher price and increases demand comparable to the un-parboiled grain, as end users demand for physical, cooking and eating qualities of parboiled rice achievable only by steam application (Islam *et al.*, 2004; Graham *et al*., 2015; Farah *et al*., 2017). Strength of improved parboiled indigenous milled rice varieties (FARO64, FARO65, FARO66 and FARO67) from steam application is shown in Figure 1. Steam application was significant on the strength of the rice studied, as FARO65 steamed for 20mins had higher strength value (205.01N) compared with 61.53N for the control sample of same variety, and other treated samples. The higher energy value observed for the sample steamed for 20mins was not different from that of Marshall *et al.* (1993) that stated that parboiling increases hardness of rice, thus given higher head rice recovery, which may be attributable to the gelatinization and compactness of endosperm starch of parboiled rice that hardens rice kernel (Yuji *et al.,* 2001).

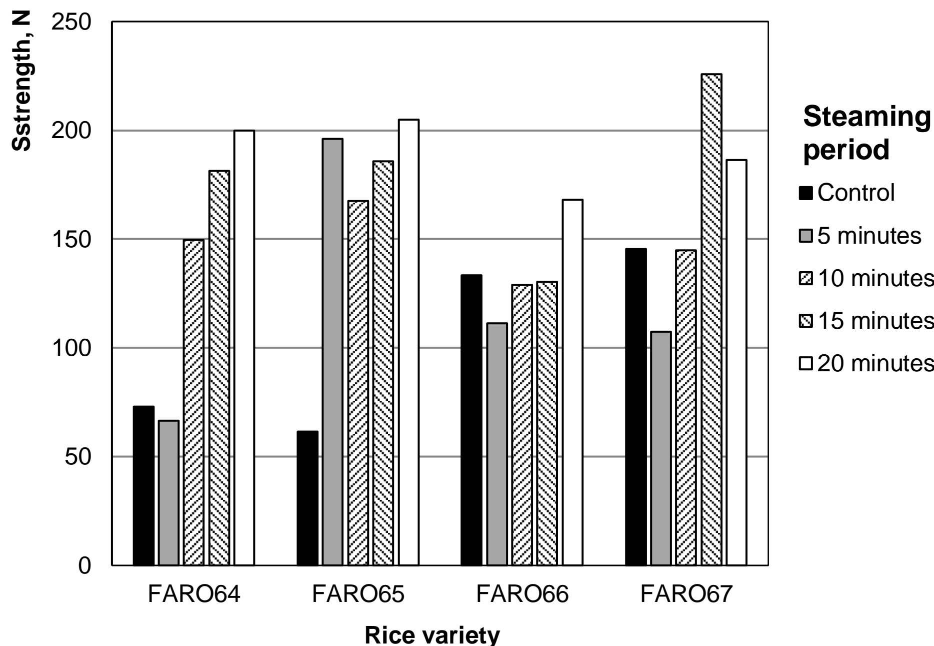

Figure 1. Effect of steaming-time on the strength of parboiled milled rice samples

For FARO64, it was obvious that steam had great effect as the strength increases with steaming, though at 5mins steaming, it was lesser than that of the un-parboiled, probably the sample may be adjusting to the steaming effect or genetic make-up of the variety. FARO65 samples steamed for 5-20mins were not significantly ($p<0.05$) different in strength from one another just as Ebrahim *et al.* (2015) reported that slightly different energies are required to break rice samples steamed between 2-10mins. Though slightly different energies would be required, they are not significantly different, but significantly different from the controlled sample. In FARO64, strength significantly ranged from 199.87N in 20mins steamed sample to 66.46N in 5mins steamed sample. Sample steamed for between 10 and 20mins were significantly different ($P<0.05$) from the samples steamed for 5mins and the control (73.06N). The expectation, according to Houssou *et al.,* 2016, is to increase strength and harden grain with steaming process. However, short time steaming and low gelatinization process weakens starch cells and reduced grain strength (Ebrahim *et al.,* 2015), and according to Islam *et al.,* 2000, energy is required to break rice sample. Energy reported for samples steamed between 10 and 20mins were 149.46N, 181.37N and 199.87N.

For FARO67, the strength varied significantly ($p<0.05$) from 107.39 to 225.83N for treated samples, though the un-parboiled sample had energy 145.54N, which was higher than that steamed for 5mins. Grain strength/hardness of samples steamed for 15 and 20mins were higher than the control sample, which was not totally in agreement with the reports of Ebrahim *et al.,* 2015 and Islam *et al.,* 2000. Rice strength is important because rice hardness facilitate high head rice yield and minimized breakages during milling, ultimate goal of rice processors and millers.

FARO66 sample steamed for 5mins had the lowest hardness value (111.40N), attributable to weakness of starchy endosperm resulting from the effect of melting during initial gelatinization, and/or partial gelatinization. It was equally noticed that samples steamed for 10 and 15mins, as well as the control were significantly not different from one another, defying the report of Ebrahim *et al.,* 2015 and Islam *et al.,* 2000. Rice with low energy has low resistance to breakage during milling leading to low percentage of head rice yield, which is of poor economic value and as such, FARO66 could be categorized as such. Steaming beyond 10mins, and up to 20mins, increased hardness value from 111.40N to 167.99N, supporting the report of Adekoye *et al.,* 2014 that hardness of improved rice varieties increases with increase in steaming-time leading to extended shelf life. Hardness of the grains was not identical probably due to genetic make-up or agronomic properties of the varieties.

Effect of steaming-time on physical properties of parboiled milled rice

Rice is mostly consumed as whole grain, and as such, its dimensions such as length, width and thickness are considered, because of their importance in developing new rice variety for commercial production and selection preference by consumers (Adekoni *et al.*, 2014). A significant increase ($P<0.05$) in length of FARO64 was observed, with 6.54mm, 6.73mm, 7.57mm and 7.30mm respectively for samples steamed for 5 to 20mins, while un-parboiled sample was 6.05mm (Table 2). Samples steamed for 5 and 10mins were not significantly different from each other but different from the un-parboiled/control sample. Likewise, samples steamed for 15 and 20mins were also not significantly different ($P<0.05$) from each other, but significantly different ($P<0.05$) from the un-parboiled sample. Based on IRRI (2009) grain dimensional classification, it was discovered that FARO64 variety changed from medium to long grain size with steaming-time. FARO65 was significant for length. The value increased from 6.02 to 6.90mm in the treated samples, as against 5.63mm recorded for control sample. Sample steamed for 10 to 20mins were not significantly different from one another, and longer than the other two, but 5mins steaming was shorter, implying that steaming increases length of FARO65, and could be termed a medium sized grain after parboiling. Significant difference ($p<0.05$) was not observed in the length of FARO66 treated samples. The mean length of the steamed samples ranged between 6.67 – 7.17mm, while the control sample was 6.33mm. The results were similar to that reported by Danbaba *et al.* (2016) on the processing characteristics, grain quality and end-use quality of hybrids and improved rice. Sample steamed for 15mins had the highest mean value, while that steamed for 5mins had the least length (treated). A reduced value of 6.90mm was recorded in the sample steamed for 20mins, and consumers prefer long grains. As a result, FARO66 should be steamed for not more than 15mins for optimum length. In FARO67, no significant difference ($p<0.05$) was observed for length (treated samples). During steaming, the length increased with increase in steaming-time (7.23, 7.53, 7.58 and 7.67mm) as against 6.79mm recorded for un-parboiled sample, similar to that of Amaka *et al.* (2014) on TOX 3145 rice grains. Sample steamed for 20mins had the highest mean value of 7.67mm, making FARO67 increase in length with increased steaming-time. Though it increased with steaming time; it was not as long as pausa Basmati 1121, which was 8.3mm (Kumar 2013).

The width value of FARO64 samples were not significantly different ($P<0.05$) from one other (treated and untreated). Steaming between 10 and 20mins resulted in slight increase in width from 2.57 to 2.62mm in the samples. FARO65 samples were equally not significant for width (treated and untreated). Highest width value (2.61mm) was recorded in the samples steamed for 15 and 20mins, while un-parboiled and 5mins steamed samples were slightly

less, meaning that steaming does not really affect width, but steaming between 15 and 20mins maximum should be adopted. The width dimension of FARO66 ranged from 2.31–2.61mm and showed significant differences ($p<0.05$). Samples steamed for 5mins and the control were not significantly different from each other, but were significantly different ($p<0.05$) from samples steamed for 10, 15 and 20mins. However, samples steamed between 10 and 20mins were different from one another.

Significant differences ($P<0.05$) were observed for the width values of FARO67 samples, with values ranging from 1.96 – 2.37mm. Un-parboiled sample had a width value of 1.98mm, which was not significantly different from the sample steamed for 20mins, samples steamed for 5 and 15mins were equally not different from each other, while the sample steamed for 10mins was different from the other samples. The width of FARO67 did not follow the pattern of the other varieties, but erratic.

Ratio of length to width of rice grains is an important attribute in shape determination (Joseph *et al*., 2015; Anounye *et al*., 2016). The length/width ratio of FARO64 was not significantly different ($p<0.05$), with values between 2.37 – 2.89mm. With 5mins steaming, length/width ratio was 2.61 compared to 2.37 recorded for un-parboiled sample. Samples steamed for 15 and 20mins had highest ratios (2.89 and 2.81), which was within the range of 1.71-3.56mm reported by Joseph (2015) for some varieties of African rice. From the results, there was no significant change in the shape of the treated and untreated samples of FARO64, as they all maintained medium shape (IRRI, 2004). Length to width ratio of FARO65 showed significant difference ($P<0.05$). It increased with increase in steaming-time and ranged between 2.35 to 2.64 for steamed samples, but un-steamed sample had 2.30. Fofana *et al.* (2011) reported a slender shape for Benin Republic rice samples with ratio of 3.51, while improved varieties were reported to have medium shape with ratio of 2.69. Base on this, FARO65 could be classified as medium shaped and does not change with steaming-time just as FARO64 varieties. Length/width ratio of FARO66 samples were not significantly different ($p<0.05$), however, highest ratio (2.88) was recorded with sample steamed for 5mins, while the lowest was 2.64 recorded in the sample steamed for 20mins, as against 2.74 recorded for un-parboiled sample. The length to width ratio of FARO66 reduced with increase in the steaming-time during parboiling except from 5 t0 10mins, though maintained medium shape throughout the steaming-periods. The values recorded were lower than 2.96 reported by Kumar (2013). Length to width ratio of FARO67 samples were significantly different ($p<0.05$) with steaming-time. Values of length/width ratio ranged from 2.58-3.56, with the sample steamed for 20mins recording the lowest ratio (2.58), while the un-parboiled sample had the highest (3.56). Samples steamed for 5mins had the highest ratio (3.45) among the steamed samples, a value similar to 3.51 reported by Anounye *et al.* (2016) in un-parboiled sample of FARO46 (slender shaped), while *OFADA* had length/width ratio of 2.35-2.44. As a result, FARO67 could be said to change shape with increased steaming from slender to medium.

Grain thickness level helps in designing milling machine, sieve selection and the calculation of milling power (Varnamkhasti *et al.,* 2008). Thickness of FARO64 variety ranged from 1.76 to 2.03mm, with 20mins steamed sample having the highest value (2.03mm), and the lowest value (1.76mm), was observed in the sample steamed for 5mins, though not significantly different from one another. It could be said that thickness of FARO64 rice variety increased with increased steaming-time. Un-parboiled FARO65 sample was 1.71mm thick, while lower values of 1.62mm, 1.59mm and 1.69mm were recorded for samples steamed between 5 and 20mins. For better percentage thickness of FARO65 variety, steaming for not less than 15mins during parboiling should be adopted.

Table 2

Effect of steaming-time on dimension of parboiled milled rice

Samples	Rice variety	Steaming period	Length	Width	Breath	Length/width ratio	1000 grain weight of parboiled milled rice
64	FARO64	Non parboiled	**6.05 ±0.01[d]**	**2.55 ±0.01[a]**	**1.89 ±0.00[ab]**	**2.37 ±0.00[c]**	**15.67 ±0.02[d]**
564		5	6.54 ±0.01[c]	2.51 ±0.00[a]	1.76 ±0.00[b]	2.61 ±0.00[bc]	24.00 ±0.10[a]
1064		10	6.73 ±0.00[bc]	2.57 ±0.00[ab]	1.79 ±0.00[b]	2.62 ±0.00[c]	18.67 ±0.06[c]
1564		15	7.57 ±0.00[a]	2.60 ±0.01[a]	1.87 ±0.00[ab]	2.89 ±0.01[bc]	18.00 ±0.00[c]
2064		20	7.30 ±0.04[ab]	2.62 ±0.00[a]	2.03 ±0.01[a]	2.81 ±0.01[bc]	17.67 ±0.06[c]
65	FARO65	Non parboiled	**5.63 ±0.02[e]**	**2.45 ±0.00[ab]**	**1.71 ±0.01[b]**	**2.30 ±0.01[d]**	**15.00 ±0.00[d]**
565		5	6.02 ±0.02[d]	2.57 ±0.00[a]	1.62 ±0.00[bc]	2.35 ±0.01[d]	16.00 ±0.00[h]
1065		10	6.63 ±0.02[bc]	2.58 ±0.00[a]	1.59 ±0.00[c]	2.57 ±0.01[c]	15.83 ±0.03[d]
1565		15	6.77 ±0.00[bc]	2.61 ±0.00[a]	1.69 ±0.00[bc]	2.59 ±0.00[c]	16.00 ±0.00[cd]
2065		20	6.90 ±0.01[b]	2.61 ±0.00[a]	1.69 ±0.00[bc]	2.64 ±0.00[c]	16.93 ±0.01[cd]
66	FARO66	Non parboiled	**6.33 ±0.01[c]**	**2.31 ±0.00[b]**	**1.57 ±0.00[c]**	**2.74 ±0.01[bc]**	**17.33 ±0.06[c]**
566		5	6.67 ±0.02[abc]	2.32 ±0.00[b]	1.26 ±0.06[d]	2.88 ±0.01[bc]	16.00 ±0.00[cd]
1066		10	7.07 ±0.00[ab]	2.49 ±0.00[a]	1.61 ±0.00[bc]	2.83 ±0.00[bc]	18.00 ±0.00[c]
1566		15	7.17 ±0.00[ab]	2.55 ±0.00[a]	1.63 ±0.00[bc]	2.81 ±0.00[bc]	15.67 ±0.06[d]
2066		20	6.90 ±0.01[b]	2.61 ±0.01a	1.64 ±0.00[bc]	2.64 ±0.01[c]	15.00 ±0.00[d]
67	FARO67	Non parboiled	**6.97 ±0.02[b]**	**1.98 ±0.00[d]**	**1.94 ±0.00[a]**	**3.56 ±0.01[a]**	**21.67 ±0.06[b]**
567		5	7.23 ±0.02[ab]	2.10 ±0.01[c]	2.00 ±0.00[a]	3.45 ±0.02[a]	22.00 ±0.00[b]
1067		10	7.53 ±0.03[a]	2.37 ±0.01[b]	1.99 ±0.00[a]	3.19 ±0.02[b]	21.50 ±0.05[b]
1567		15	7.57 ±0.03[a]	2.23 ±0.01[c]	1.99 ±0.00[a]	3.42 ±0.02[b]	21.00 ±0.00[b]
2067		20	7.67 ±0.03[a]	1.96 ±0.00[d]	1.94 ±0.00[a]	2.58 ±0.19[c]	16.33 ±0.06[cd]

Effect of steaming duration on the thousand grain weight (1000gw) of milled rice

Thousand grains weight of rice measures the net weight of a randomly selected 1000 grains. It is important in evaluating rice yield and varietal differences, as well as a great impact on seedling viability and growth, though, correlated to kernel width, thickness and shape (Prasad *et al.*, 2016). Un-parboiled sample had the lowest grain weight (15.67g), while sample steamed for 5mins weighed 24g. However, samples steamed between 10 and 20mins had decreased weight, corroborating the report of Gayin *et al.*, 2009. However, for FARO65 variety, samples steamed for 20mins recorded the highest thousand grain weight (16.93g), 5mins steamed sample and control weighed 16g and 15g respectively, with significant differences within the samples. Pore sealing during steaming might have increased the weight (Prasad *et al.,* 2018), and thus, increases rice thousand grain weight. Samples steamed for between 5mins were significantly different from samples steamed from 10 to 20mins.

A significant difference ($p<0.05$) was observed in the grain weight of FARO66 samples, with the result ranging from 15-18g. Sample steamed for 20mins recorded the lowest weight (15g), while 18g was recorded in samples steamed for 10mins. Un-parboiled sample weighed 17.33g. The values did not follow same pattern with increasing steaming-time, making it different to what was recorded in FARO65, though the mean values were not far from the range of 19.86-21.65g reported by Singh *et al.* (2002) and Prasad *et al.* (2018) in the un-parboiled and parboiled samples steamed for 40mins. Thousand grains weight of FARO67 samples ranged between 16.33 and 22g, and was similar to what was recorded in FARO66. The control sample and samples steamed for 5 to 15mins were significantly different ($p<0.05$) from that steamed for 20mins. Un-parboiled (control) sample weighed 21.67g, and was noticed that steaming from 10 to 20mins caused reduction in weight of the parboiled rice variety. To achieve a weighty grain in FARO67 variety, steaming for short periods was better. Also, reduction in grain weight might be due to poor pore fillings with water molecules as reported by Singh *et al.* (2002).

Conclusions

It should be noted that the proximate composition of these new rice varieties was comparable to those of existing rice samples worldwide, the study, however, looked at some parameters used to assess rice quality. Though some of the results were outside expectations, however, it can be concluded that steaming, as a unit operation in rice parboiling, has significant impact on the colour of parboiled milled rice, and improve the strength required to break parboiled rice during milling. Likewise, steaming period positively influenced rice grain dimension and quality, as witnessed in the varieties, where medium shaped ones changed to very long size when steamed for 15mins, though sample size was not considered. For a good quality milled rice grains, rice properties must be considered during processing, as every rice variety has optimum processing requirement. However, in general, steaming for 15mins gave the best result, and thereby recommended.

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Influence of pectin-based and modified starch thickeners on the structural characteristics of low-calorie apple jam

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Abstract

Introduction. The aim of the study is to determine the effects of thickeners on the structural characteristics of low-calorie jam.

Materials and methods. The technology of apple puree jam with complete removal of refined sugar and with the addition of sweetener was studied. Research methods: calcium-pectate method for determination of pectin substances, refractometric method for determination of dry substances, method for determining the mass of the load to destroy the structure of the jam to determine its strength; profile method on a 10-point scale of correspondence of intensities of sensation of aromatic and taste properties.

Results and discussion. Removal of refined sugar from the recipe leads to deterioration of the structural characteristics of the jam. In order to determine the optimal quantitative ratios of ingredients, the dependence of physicochemical quality indicators of jam on the content of thickeners in it.

Reduction in the amount of sugar in the range of 100–333 g / 1000 g leads to a decrease in strength from 320 g to 160 g. At the same time, a significant amount of dry matter is lost: from 65,2 to 45,2%. Their number partially increases with increasing content of apple puree from 550 g / 1000 g of jam (control sample) to 650 g.

This explains the slight increase in titratable acids content from 1.0 to 1.5 mg / 100 g. The titratable acidity was main when choosing pectin as a food additive to improve the structural characteristics of the finished product. A sufficient amount of pectin can be considered 10 g / 1000 g of jam. The strength of the jam's structure at this amount is 300 g. The addition of modified corn starch Regel 200 G in the amount of 10 g /1000 g of finished product allows to stabilize the texture of the jam and maintains its strength..

The addition of stevia extract in the amount of 15 g / 1000 g is optimal. Further increase in the amount of stevia extract is not justified, because the taste remains intensely sweet and the difference is not particularly noticeable. The taste profile of the studied samples shows the preservation of sweet taste and a slight increase in the fullness of bitter taste.

Conclusion. Adding pectin and starch as thickeners to jam, which contains dry stevia extract and does not contain refined sugar allows to get low-calorie jam and preserve the sensory properties of the product.

Introduction

The healthy food has led to interest in low-calorie food products (Manippa et al., 2021). There is a modern trend in restaurant business for development of food products sugar-free. Fruit gel is an intermediate moisture food containing fruit pulp, sugar, pectin, and acid. For a this product like jam, it is important to understand the relationships between the sensory perception of food gel and textural or rheological properties.

A new product like a low calorie fruit gel in which the sugar content is low has to be manufactured by controlling the sensory and textural perception of the product. The chapter describes the rheological behavior of fruit gels as influenced by alternative sweeteners by partial or full replacement of sucrose (Basy et al. 2021).

Confectionery is not an essential product, but it is in high demand. Among these products are jams, which together with other canned fruits and berries are a source of pectin and only organic ingredients, vitamins and minerals (Oliver, 2015; Wim et al., 2002). This biologically active substance has medicinal properties, has antibacterial properties (Wikiera et al. 2014). To make fruits available for consumption during off-season, the fruits are processed into shelf-stable products like jam (Shinwari et al., 2018). That is why this work proposes developed technology low-calorie jam.

Many works are devoted to the study of technologies and components in the production of jams and other fruit and berry products (Rafeek et al., 2015). One of the main characteristics of the jam is a jelly-like consistency. It is due to the interaction of sugar with pectin in the presence of a significant amount of organic acids (Figuerola et al., 2007).

According to the formula (Cervera-Chiner et al., 2021), the sugar-free jam is prepared from water, strawberries, pectin, maltodextrin, citric acid, sodium citrate and sucralose. The sugar-free jam has the advantages of reasonable formula, advanced process, healthy and nutritional product and the like.

Stevia is of great scientific and practical interest in the production of low-calorie foods and which, in addition to the formation of sweet taste, gives a functional focus to products using it (Muñoz-Almagro et al., 2021). Stevia leaf extract is zero-calorie. It is being used globally to reduce energy and added sugar content in foods (Ashwell et al., 2015).

However, the substitution of sucrose by stevia, as sweetening agent, could be a challenge for industry, since in addition to the sweet taste, other sensory features could modify the final product (Reale et al., 2020). Stevia gives a functional focus to products using it.

The sweet secret of stevia is a complex molecule called stevioside, which is a glycoside. It is this complex molecule and a number of other related substances that are responsible for the extraordinary sweetness of stevia (300 times sweeter than sucrose). Stevia extract is not fermented by microorganisms and does not stain jams. Overall, stevia shows promise as a new tool to help achieve weight management goals.

The importance of good practice in the use of stevia in foods has been described both as a potential health product and a sweetener (Wang et al., 2021). Based on recent reports, this work is considering adding stevia extract to the jam.

The aim of the study is to determine the effect of thickeners on the structural characteristics of low-calorie jam, to stabilize the texture of the jam, maintain the strength characteristic of the classic jam-like consistency

Materials and methods

Materials

The study used model samples jam with sugary and sugary-free; puree from apple. Highly esterified apple's pectin brand WEJ-5 manufactured by Rektowin. Its physicochemical characteristics: gel strength 100±5 ° SAG, degree of esterification 50–55%, pH of 1% solution 3,4±0,5.

Preparation of research samples

Preparation of apple puree: sorted apple fruits are washed in cold water, crushed and cooked in a special dish until completely softened (the duration of the technological stage is not more than 15 minutes); grind through a special wiping sieve with a hole diameter of not more than 1,5 mm.

Preparation of jam: apple puree is boiled, stirring constantly; add the required amount of sugar / sweetener, pectin concentrate solution and starch (duration of the technological stage 10–15 minutes).

Packing: puree in a hot state at a temperature of 95–97 °C is packed in a glass container; glass jars with jam are covered with lids and subjected to sterilization (duration of sterilization of jam in glass jars with a capacity of 0,5 liters – 15 minutes, 1,0 liters – 25 minutes); then the jars are sealed, turned upside down and cooled (Bekele et al., 2020).

Experimental plan

The study of the effect of a new food supplement with other ingredients requires further step-by-step research. The jam's recipe was supplemented with sweetener and gradually. Table 1 shows various ingredients for making jam.

Table 1

Ingredient composition of model samples

№	Ingredient	Mass, g						
		Sample (control)	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**	**Sample 6**
1	Puree from apple	550	600	610	620	630	650	650
2	Water	110	285	366	350	325	305	305
3	Sugar	333	100	-	-	-	-	-
4	Stevia extract	-	5	10	15	20	25	30
5	Apple's pectin	7	5	7	10	15	20	30
6	Modified starch	-	5	7	10	15	15	15

Description of methods

Methods for determining dry soluble substances

Refractometric method is to determine the mass fraction of dry matter in the product by the refractive index of its solution (Chen, 2019). For this purpose two drops of sample of model samples put on a prism of a refractometer. The sample was left in the refractometer to equilibrate thermally for two minutes and then the reading was performed. Temperature corrections are used to bring the refractometer reading to 20 °C. The repeatability determination was evaluated performing 10 subsequent measures under repeatability conditions on the same samples (Giulio et al., 2008).

Method for quantitative determination of pectin substances

This method including the conversion of pectin into a solution, demethoxylation with alkali and treatment of the resulting pectic acid solution with a calcium compound. The treatment of the pectic acid solution is carried out its hydroxide by titration with calcium in the presence of 0.002 wt. including copper acetate and including sodium chloride per 1 wt. including pectin (Yu. et al., 2021).

Mass fraction of pectin in the sample X,% was determined:

$$X = \frac{10^4 m_2 v \cdot 0.9235}{m v_1 \, (100 - W)} \qquad (1)$$

where m_2 – the mass of the precipitate is calcium pectate,g; v – total amount of extract, cm^3; 0,9235 – conversion factor of calcium pectate; m – the mass of the product, g; v_1 – the amount of extract taken to determine pectin, cm^3; W – mass fraction of water in the product,%.

Method for determining the strength of the jam

The method is based on determining the mass of the load required to destroy the structure of the gem. The essence of the method: a sample of 30 ml in a cylindrical container is subjected to destruction by loading at a rate of 10–15 g/min. The mass of the load, which was critical, was recorded.

Expert method of sensory evaluation

The expert method of values quality indexes determination is based on committing the thought of the highly skilled and experienced specialists-experts (The expert of − it a specialist on the certain type of object which owns the increased sensitiveness to properties of this object) (Kuzmin et al., 2020a). The intensity was evaluated on a 10-point scale.

Results and discussions

Acording the objectives of the study, it is proposed to make some samples. One of them contained sugar and complied with regulations. The remaining samples 2–6 were prepared without sugar. Based on the results of modern scientists, it was decided to add dry extract of stevia leaves as an additional source of sweet taste (Roik et al., 2015). Modified starch and

pectin were included in the recipe to ensure normalized physicochemical quality indicators (Dangi et al., 2021).

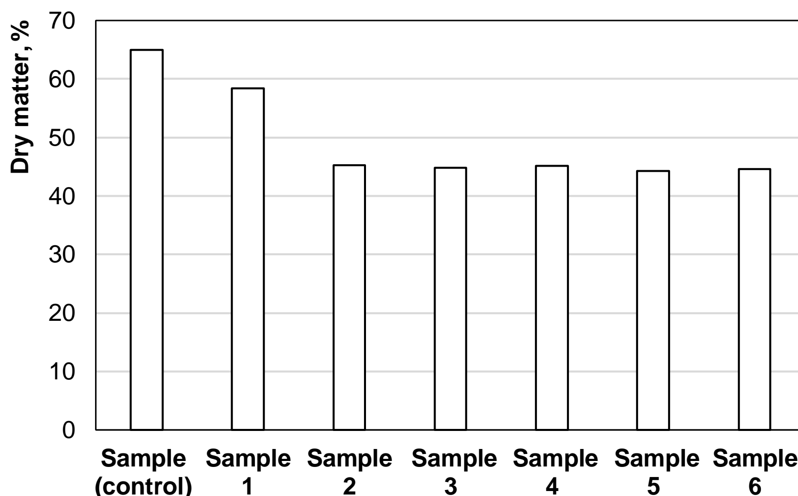
The creation of new low-calorie jam provides for the following:

– Justification for the use of stevia extract in the production of jam based on apple puree;
– Reduction of energy value of the product due to removal (reduction) of sugar;
– Replacement of refined sugar with stevia extract while maintaining consistency by adding pectin and modified starch;
– Determination of physico-chemical quality indicators of the developed product;
– Determination of sensory assessment of the quality of test specimens according to regulatory documentation.

When adding a new ingredient to the recipe, it is necessary to determine the optimal mass and method of application. The results obtained from the samples were compared to show the differences among them. Especially important determine at which technological stage the application of food additives will have the best results.

Dry matter content

The Figure 1 shows that the reduction in the amount of refined sugar (sample 1) and its extraction (samples 2–6) leads to a decrease in the amount of dry matter, which can lead to loss of structure of the jam. Increasing the amount of stevia extract from 5 g to 30 g per 100 g of product affects the dry matter content very little. This means that you need to conduct a sensory evaluation of the quality of the finished product.



Analysis of titrated acidity of samples

The addition of various amounts of apple puree in the samples helps to increase the mass fraction of titrated acids due to organic acids that are part of fruit puree (Figure 2). That will allows extending the shelf life of jams and prevents microbiological spoilage (Shinwari, 2018).

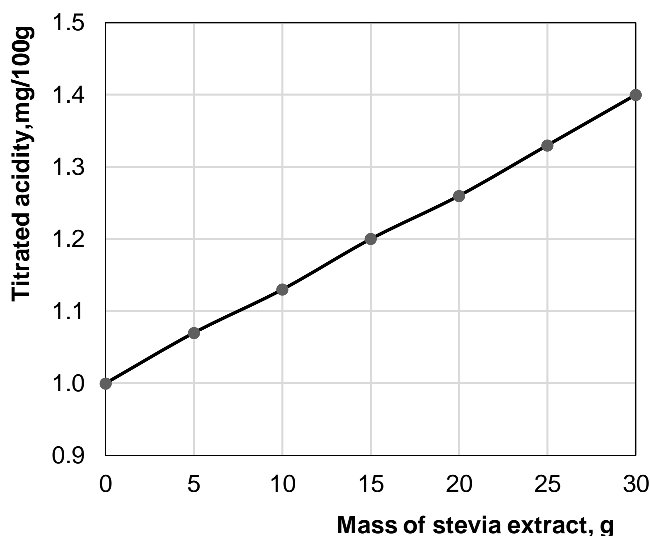


Figure 2. Dependence of mass fraction of titrated acids on stevia content

The titrated acids found in this study was in the range of 1.0–1.5 mg/100 g, showing significant differences among the acidity of six samples. This is due to the increase in the amount of apple puree of 550–650 g per 1000 g (Table 1). In jams with a mild sour taste and high pH, pectin should be used. Especially if the dry matter is 60 – 68 %. If you use very sour fruits and berries or use a lot of acid to taste, low pH requires medium or slow pectin. Quick-release pectin is able to gel even when the dry matter content is 55 %, but sometimes it is used when the dry matter content is 55–60 % (Bekele et al., 2020).

Strength of samples

Strength is the most important indicator for culinary dishes with a jam-like structure, which determines most of their organoleptic characteristics. Strength is studied at the end of the process of structure formation of the gel, which lasts depending on its prescription composition and the concentration of the main prescription components. According with data Krzysik et al., (2020) highly esterified apple pectin of the WEJ-5 brand manufactured by Rektowin was selected. The results of the study of strength are shown in Figure 3.

The figure shows that the strength of the studied samples of jam is in the range of 160–330 g. The highest value of strength reaches samples 4–6. This amount of the pectin (15–30 g) is not acceptable enough for apple jam, which naturally contains a significant amount of pectin. It does not meet the daily needs of pectin consumption (Muñoz-Almagro et al., 2020). We consider optimal sample 3, the strength of which is 300 g, pectin – 10 g/1000 g. This value lies in the rational range of strength of control samples with the addition of sugar, which is 320 g.

However, pectin is a rather expensive component and is strictly regulated in terms of content, so it is advisable to replace some of it with modified starch. Oxidized starches are of the greatest interest in the production of restaurant establishments.

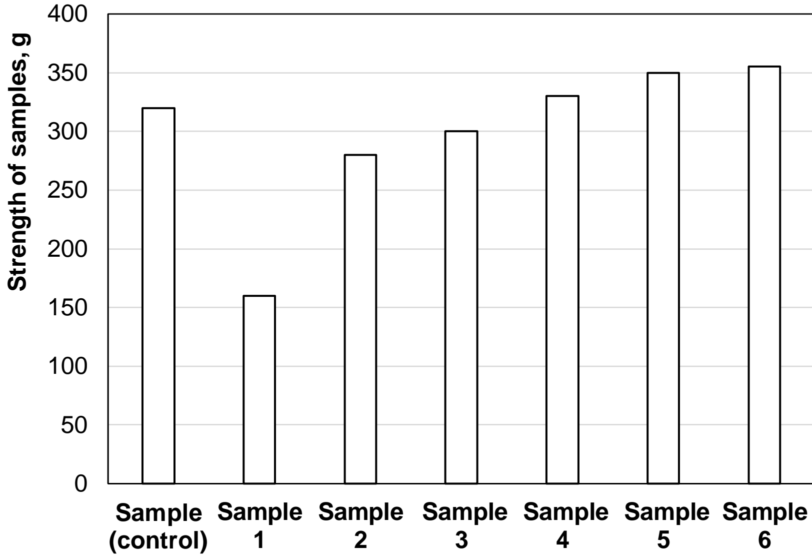


Figure 3. Dependence of strength of samples

The next step is study of the degree of swelling of the modified starch (Figure 3).

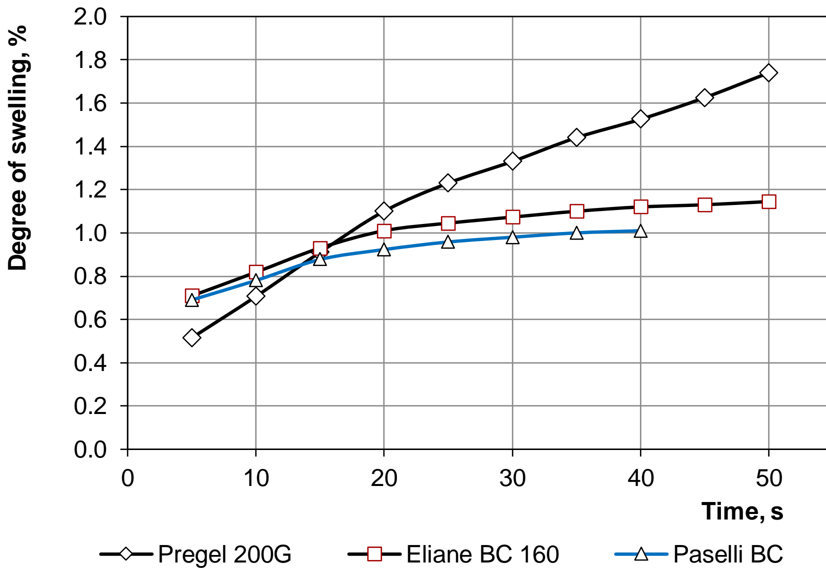


Figure 3. Changing the degree of swelling of the modified starch depending on the duration of swelling

It was determined that the greatest degree of swelling has a modified corn starch Regel 200 G – 1,75%.

Hydration function of modified starches (Table 2) is its main characteristic when choosing.

Table 2

Degree of hydrophilicity of modified starches

Name of the hydrocolloid	**Hydrophilicity**, %
Potato elianebc 160	36,7
Potato pasellibc	25,9
Corn Regel 200 G	43,5

Thus, based on the technical data of the manufacturer, it is found that the optimal is modified corn starch Regel 200 G hydrophilicity of which is 43,5%. Using the latter will help create heat-reversible gels and reduce the amount of sugar in the products.

Through theoretical calculations and experimental studies, it was possible to establish the optimal recipe of the jam, which includes stevia extract powder, apple pectin and modified starch. This is the sample 3.

Formation of the quality of the jam

According to organoleptic indicators, jams must comply with sample control and some the indicators (Bekele et al., 2020). During the preparation of jam based on apple puree, a sensory evaluation of the obtained samples was performed. The essence was to decompose the taste into simple components (sweet, salty, bitter, spicy, sour, tart) intensity. Figure 4 shows the profile of samples 1, 2, 3, 4 and control.

The taste of stevia extract remains intensely sweet and the difference is not particularly noticeable. It was found that the closest to the control sample is the sample 2. This means that the optimal concentration of sweetener that can be introduced is 10 g per 1000 g of finished product. The subsequent increase in the amount of stevia extract leads only to the deterioration of flavoring properties.

The characteristics of the test samples are presented in Table 3.

Table 3

Characteristics of the model samples

Characteristic	**Sample control**	**Sample 2**
Appearance	Inherent in jam, transparent homogeneous mass	Light yellow homogeneous mass
Color	Inherent in apple jam, has a light yellow hue	Inherent in apple jam, has a yellow tinge
Taste	Gentle, sweet	Pleasant, tender, sweet with bitterness
Scent	Inherent in apple jam	Inherent in apple jam
Consistence	Homogeneous puree, well spread	Homogeneous jelly-like mass, does not spread on a horizontal surface

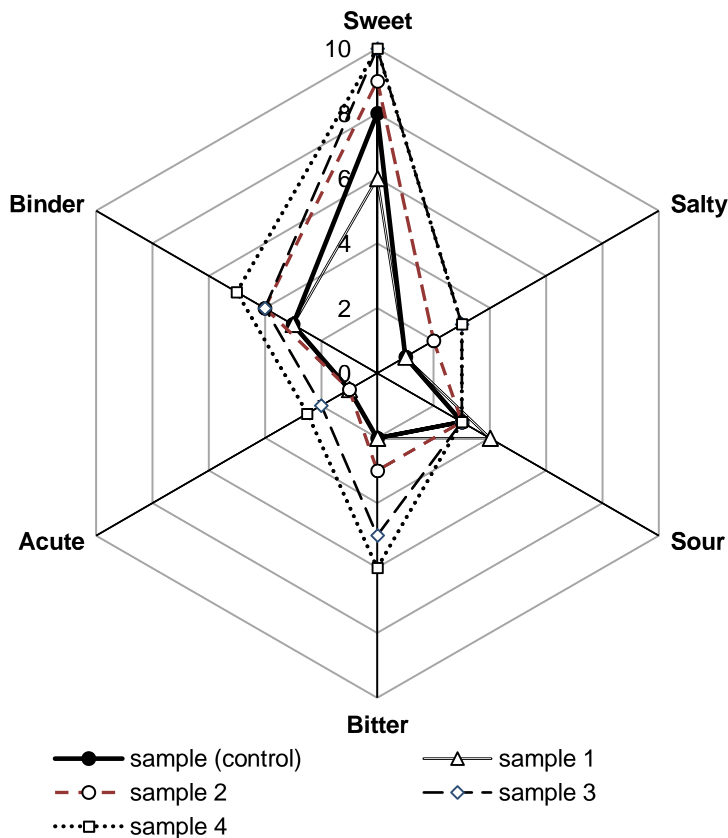


Figure 4. Sensory profilograms of samples

The main purpose of this work was to reduce the energy value and avoid refined sucrose. For this purpose, the search for the optimal recipe that will preserve the physical and chemical quality indicators.

Jam is mainly a source of carbohydrates (48–65%) and its energy value is 183–253 kcal/100 g. From biologically active substances emit ascorbic acid. Its amount is depending on the type of raw material and cooking technology ranges from 1,5 to 53,3 mg per 1000 g of product. Non-sterilized jams include more ascorbic acid than sterilized ones.

Despite all this, the main goal of this work was to reduce the energy value and avoid refined sucrose. For this purpose, the search for the optimal recipe that will preserve the physical and chemical quality indicators.

Analysis of the results of the calculation of energy characteristics shows that the main objective of the work has been achieved: the energy value of the low-calorie has been reduced to 123 kcal. It is slightly less than 2 times compared to apple jam (sample control).

Table 4

Nutritional value of low-calorie jam

	Daily norm	Sample control	Low-calorie jam
Energy value, kcal	1684	250	123
Proteins, g	76.0	0.4	1.6
Carbohydrates, g	219.0	65.0	29.0
Dietary fiber, g	20.0	2.3	4.3
Water, g	22.7	32.9	63.9

	Part of the norm in jam in 100g, %
Energy value	14.8
Proteins	0.5
Carbohydrates	29.7
Dietary fiber	5

Conclusions

1. According to the task of scientific work, it was studied influence and value of thickeners on the structural characteristics of low-calorie jam. Complete replacement of sugar with dry stevia extract in the recipe of low-calorie jam was made. It was found that the optimal concentration of sweetener is 10 g / 1000 g of finished product.
2. Theoretical calculations and experimental studies have established the optimal recipe for the low-calorie jam, which includes stevia extract powder, apple pectin and modified starch.
3. During creating a new jam, it is advisable to use modified corn starch Regel 200 G in the amount of 10 g per 1000 g of finished product. It helps to create heat-reversible gels and reduce the amount of sugar in the products. We consider optimal sample 3, the strength of which is 300 g. This value lies in the rational range of strength of control samples with the addition of sugar, which is 320 g.
4. Sensory evaluation of the model samples shows the preservation of sweet taste and a slight increase in the fullness of bitter taste. It was found that the closest to the control sample is the sample 3. This means that the optimal concentration of sweetener that can be introduced is 10 g per 1000 g of finished product.
5. The energy value of the low-calories jam is 123 kcal. The share of energy value that satisfies 100 g of this jam – 7,3 % of a daily requirement. Consumption of 674 g of low-calorie jam will provide the daily norm of energy value.

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Effect of flattening wheat grain on grinding modes in roller mill

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Abstract

Introduction. The flattening process is used to intensify the grinding of grain during varietal milling of wheat, while the optimal gap between the rolls of the ivy system remains uncertain.

Materials and methods. Flattening was carried out in a laboratory ivy machine with a roller diameter of 144.0 mm; 68.4 mm and a rotation speed of 14.6 s^{-1}. Grinding of wheat grain was carried out in a roller mill. The length of the rollers is 70 mm; the number of rifts per 1 cm circle of rollers is 6, the slope of the rifts is 12%, the ratio of circular speeds is 1:2, the speed of rotation of the high-speed roller is 3.93 m/s, the diameter of the rollers is 150 mm, the interdependence of the rifts is the back on the back, the angles of exacerbation of the rifts are 35°/70°. The mode of grinding wheat grain in the ivy machine and in the roller mill was determined by sifting the products obtained through a sieve with holes of 1.0 mm. Granulometric analysis of crushed products was determined by sifting on a standard set of sieves with module $\Delta \approx 1.21$.

Result and discussion. When grinding ivy products in a roller mill, the total yield of intermediate products is nonlinear in nature and can be approximated by the equation of the second degree. The optimal distance between the rollers of the ivy machine is 1.4 mm, provided that small products after flattening were previously isolated from the mixture of the flattened product.

The total product of intermediate products after flattening and grinding in the roller mill has a complex nonlinear nature, which did not allow to establish the optimal value of the gap value between the rollers of the ivy system. The total product of intermediate products obtained in total during flattening and grinding is 2.9 % greater than the total product product yield obtained only in the roller mill with small ivy products previously removed.

Differential curves are polymodal and have 5 maximums. Integral curves have an S-shaped appearance. When grinding whole grain, the total yield of intermediate products is greater than when grinding flattened grain in a roller mill under the same conditions. When crushing flattened grain, a greater number of large fractions of products are formed due to small ones.

Conclusion. In the range of the established optimal clearance between the rolls of the flattening machine, there are no significant differences between the two methods of grinding wheat.

Introduction

Intensification of the process of grinding wheat grain into flour is one of the urgent scientific problems, the solution of which is devoted to a large number of scientific works. (Dmitruk et al., 2013; Fistes et al., 2009; Mousia et al.,2004; Netrebsky, 2006; Warechowska et al., 2016).

The introduction of BUHLER technology in the twentieth century led to the failure of a number of technological methods of processing wheat grain into varietal flour. With a reduction in the technological process of grinding wheat grain into flour, it became necessary to find methods for intensifying the grinding process and increasing the output of finished products. (Kharchenko et al.,2016). One of these methods is the preliminary flattening of wheat grain before sending it into a dredged process (Morhun et al.,2010; Shutenko et al.,, 2014b).

Grain resistance to destruction is determined by complex processes that depend on the nature and condition of the grain, as well as the mode of mechanical load. The nature and condition of the grain should include such grain indicators as humidity, vitreity and size. The load mode must include the conditions of destruction: the size of the gap between the rollers, the surface of the rollers, the ratio of the circular speeds of the rollers, etc. (Dziki et al., 2012; Stefan et al., 2013; Voicu et al.,2013; Voicu et al.,2013).

Siberian V.A. and his collaborators, examining the process of flattening, concluded that the flattening of wheat grain before sending it to the first break system leads to a decrease in the yield of large grains with an increase in the degree of preliminary destruction of the grain, but recommendations on the size of the gap between the rollers of the ivy system were not provided. (Kharchenko et al., 2016; Shutenko et al., 2014a).

The main attention of many researchers was energy consumption during grinding and output of intermediate grinding products (Deineko et al.,2012; Kharchenko et al., 2016; Shutenko et al.,2014b). Despite the studies, a number of addictions remain undeseen. The main question that needs to be addressed is what should be the size of the gap between the rollers of the ivy machine to obtain the largest product of intermediate products in the roller mill I break system.

Odegov et al. (2004) recommends the gap value between the rollers of the ivy machine from 1.6 to 1.8 mm, Perekopskyi & Baranov (2004) recommends setting the gap value between the rollers 1.4 mm. Afanasj'ev, Ostrykov & Manujlov (2018) recommends for the use of flattened wheat, barley and sheep grain in feed production to set the gap value between the rollers of the ivy machine 0.5–0.6 mm. Recommended by the researchers different values of the gap between the rollers of the ivy machine do not give an unequivocal recommendation of the distance between the rollers of the ivy machine when grinding wheat grain into flour. Researchers who studied the process of flattening did not pay attention to the issue of the need to isolate small products that are formed during the process of flattening. It is known that small grain grinding products affect the efficiency of the grinding process (Kharchenko et al., 2015).

Based on the analysis of scientific works, the purpose of the research was to establish the influence of the flattening process on the product of grinding products in the roller mill and to establish the optimal value of the gap between the rollers of the ivy machine.

Materials and methods

Grain preparing

Before the start of research, wheat grain was cleaned in a laboratory grain cleaning separator in order to isolate small impurities, coarse and small grains. The purpose of this operation was also to align the grain to geometric dimensions. The grain cleaning separator used a set of lattice canvases with holes of 3.0×20 mm, 2.4×20 mm and 1.8×20 mm.

The grain fraction aligned in size was additionally passed through a laboratory aspiration channel with a width of 60 mm in order to isolate light impurities and partially pinched wheat grain. After that, the quality indicators of the experimental fraction of wheat grain were determined according to standard methods. In the prepared wheat determined the humidity, nature and glassiness of grain, as well as the mass of 1000 grains.

Before the flattening, wheat grain weighing 5 kg was poured into a container filled with a formula calculated according to the 1 (Kharchenko et al., 2018) amount of water. The final estimated moisture content of wheat was taken 15.0%.

$$G_w = G_g\left(\frac{W_1 - W_0}{100 - W_1}\right), \qquad (1)$$

where G_w, G_g – accordingly, the mass of water and the mass of grain to be moistened; W_0, W_1 – accordingly the grain moisture content is initial and set (W = 15.0%).

After adding an estimated amount of water, the grain was actively mixed for 5 min and remained for a day for lag. Before flattening, the grain was additionally thoroughly mixed. Before the start of research, the weight of wheat grain was selected to determine the actual moisture content of the grain, and the rest of the wheat grain was sent for research. Of this grain mass, 1.0±0.1 kg of wheat was left for a control sample, which was crushed in a roller mill without flattening.

Analysis of grain quality indicators

The moisture content of the experimental wheat grain was determined in accordance with ISO 712:2009(E). «Cereals and cereal products. Determination of moisture content» (ISO, 2009a), by drying in a drying cabinet crushed in a laboratory mill the weight of wheat grain weighing 5 g for 60 min. at a temperature of 130 °C.

The bulk density of wheat grain was measured using a liter purquet PB-2 in accordance with ISO 7971-3:2009(E). «Cereals – Determination of bulk density, called mass per hektolitre» (ISO, 2009b). The measured mass of one liter of wheat grain was weighed on technical scales.

The mass of 1000 grains of wheat was measured in accordance with ISO 520:2010. "Cereals and pulses – Determination of the mass of 1000 grains" (ISO, 2010) by deducting 1,000 whole grains from the total mass of wheat grains.

The vitreousness of wheat grain was determined using the DSG-3 diaphanoscope (Olis, Odesa, Ukraine), by shining 100 grains of wheat with light rays. The vitreousness was determined in twelve reposts with the following calculation of the average value (Kharchenko et al., 2017).

After preparing wheat grain for flattening, it had the following quality indicators: initial grain moisture – 13.0±0.08%; grain vitreousness – 21.3±4.7%; nature of grain – 756.6±3,0 g/l; weight 1000 grains – 38.0±0,6 g.

Method of flattening and grinding

To find out the effect of the flattening process on the process of grinding wheat grain in the roller mill, two research options were carried out.

In the first version of the studies, small products formed during the ivy process were selected in a laboratory aspiration channel with a channel width of 60 mm, as shown in Figure 1.a, in order to isolate all small products (less than 1.0 mm) and bring large ivy products to the same conditions. Purified products were sent to the roller mill for grinding.

In the second version of the study, all products formed in the process of flattening were sent to the roller mill without separation, as shown in Figure 1.b.

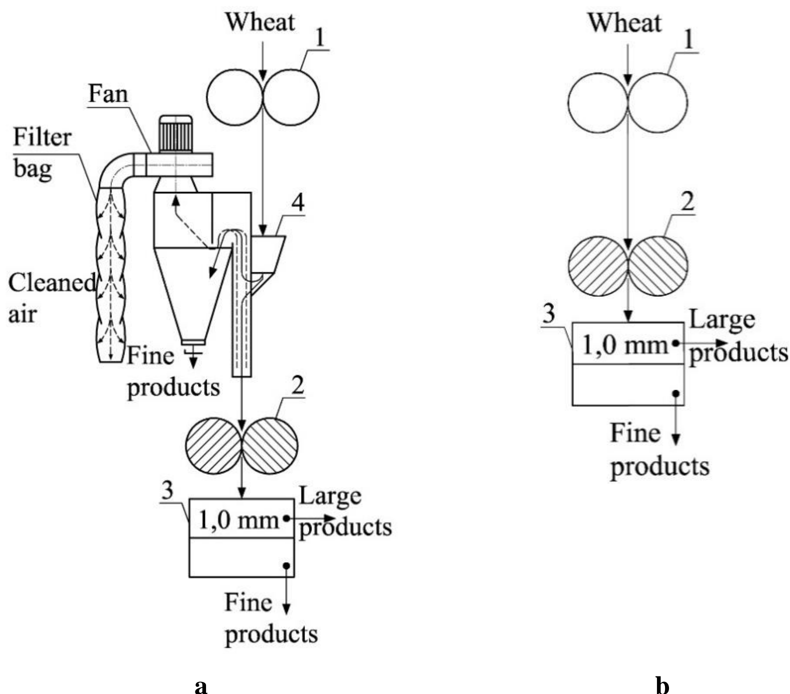

Figure 1. Schematic diagram of studies of the impact of flattening on the total product of intermediate grinding products:
1 – flacking mill; 2 – roller mill; 4 – control sieve; 4 – aspiration channel.

Flattening of wheat grain was carried out in four different modes, which were characterized by the distance between the rollers of the flaking mill. The distance between the rollers was measured using calibrated hand plates. The distance between the rollers was set from 0.4 to 1.6 mm in increments of 0.4 mm with a year of 0.4 mm. The speed of rotation of the rollers of the flaking mill was 14.6 s^{-1} (880 rpm), the width of the rollers was 68.4±0.1 mm, and the diameter of the rollers was 144.0±0.01 mm, the ratio of the circular speeds of the rollers was 1.0.

Flaking products were crushed in the roller mill of the LM-2 laboratory unit (Elelmiszeripari Gepgyar, Budapest, Hungry) without changing the gap between the rollers. Grinding products were selected using a tray in four re-sieves, sifted on a metal-rolled sieve

with holes of 1.0 mm. The passage of the sieve was weighed and listed as a percentage. The amount of the product is allocated by the passage of a sieve of 1.0 mm, expressed as a percentage determined the mode of grinding wheat grain in the roller mill.

Roller rollers had the following kinematic and geometric parameters: the length of the rollers – 70 mm; the number of rifts per 1 cm circle of rollers is 6, the slope of the rifts is 12%, the ratio of circular speeds is 1:2, the speed of rotation of the high-speed roller is 3.93 m/s, the diameter of the rollers is 150 mm, the interdependence of the rifts is dull to dull, the angles of exacerbation of the rifts are 35°/70°.

After sifting, the samples processed experimental data using Advanced Grapher software and made conclusions about changing the overall product and output of individual fractions of grinding products.

Method of granulometric analysis

After determining the total product, the products from the four repeats were combined and the granulometric composition of grinding products was determined. Granulometric analysis was carried out using a set of standard sieves with the condition that the sieve module was within Δ≈±1.21 (Chornyi et al., 2021).

Results and discussion

Effect of the gap value between the rollers of the flaking system on the product of intermediate flaking products

Studies have found that the yield of products that are formed after flattening are nonlinear in nature and can be exproximed by the exponential equation. These results also confirmed the exponential dependence gained in the work (Kharchenko et al., 2016). The results of the research are shown in Figure 2.

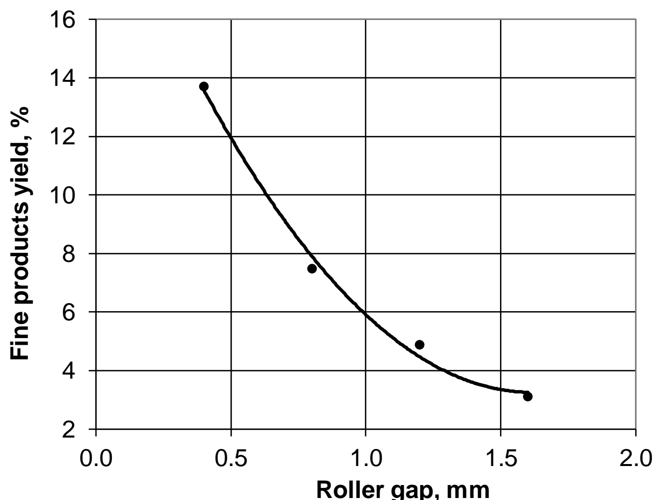

Figure 2. Yield of intermediate products obtained after flattening of wheat grain

The curve can be aproximized by the following equation:

$$Y = 21{,}24e^{-1{,}216d} \quad (2)$$

where Y – the total output of intermediates after flattening,%; d – the distance between the rollers of the ivy machine, mm.

The standard error of experimental and calculated values is 0.49%, and the correlation coefficient is 0.99, indicating a strong relationship between the studied traits.

Crushing of flattened wheat in roller mill and draped system with selected small products

The results of studies have shown (Figure 3) that with an increase in the distance between the rollers of the ivy machine, the total yield of small grinding products after the roller mill increases by parabolic dependence, which can be described by the following equation:

$$Y_{Rm} = -7{,}18x^2 + 20{,}42x + 32{,}7 \quad (3)$$

where Y_{Rm} – general output of grinding products after the roller mill,%; x – the distance between the rollers of the flaking system, mm.

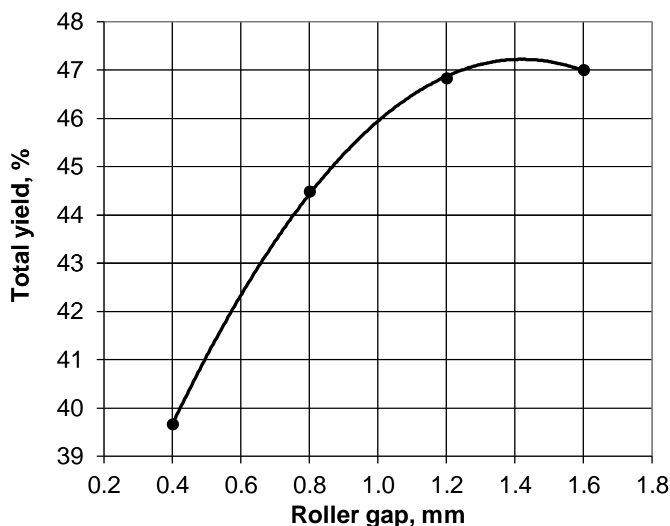

Figure 3. Effect of the distance between the rollers of the flaking system on the total output of grinding products (with selected products after the flaking mill)

The correlation coefficient is 0.99, which indicates a strong connection of the studied signs. The standard deviation is 0.05%.

This nature of the curve may be associated with the deformation of flattened wheat grain. At a distance between the rollers of 0.4 mm of the flaking mill, the greatest deformation of the grains occurs. When grinding such grains in the roller mill, the rollers will have less impact on the grain. With an increase in the size of the gap between the rollers of the ivy machine, the deformation of wheat grain will decrease, but when such wheat is crushed, the

rollers of the roller mill will increase the impact on these grains. At the same time, the resulting small products do not affect the grinding process in the roller mill, because they are allocated in the aspiration channel.

The shape of the parabolic curve has an extremum, which allows you to determine the value of the distance between the rollers at which the largest yield of intermediate grinding products after the roller mill was observed.

By differentiating equation 3 and equating it to zero, we will get:

$$\frac{dY_{Rm}}{dx} = -7{,}18 \cdot 2x + 20{,}42 \quad (4)$$

$$-14{,}36x + 20{,}42 = 0 \quad (5)$$

Solving the equation (5) we obtain the value of the gap value between the rollers of the flaking system, in which the largest yield of intermediate grinding products after the roller mill was observed:

$$x = \frac{20{,}4}{14{,}3} = 1{,}42 \approx 1{,}4 \text{ mm.}$$

The optimal value of the gap between the rollers of the flaking mill, which observed the largest yield of intermediate products when crushing flattened wheat is 1.4 mm.

Crushing of flattened wheat together with small products in the roller mill of I break system

When grinding in the roller mill of flattened wheat grain together with small fractions formed in the process of flattening, the nature of the dependence of the total yield of grinding products changes significantly. The curve of the total product of intermediate products of the two systems has a descending type. At the same time, the largest total product was observed at a gap value between the rollers of the ivy system of 0.4 mm and amounted to 63.5%. An increase in the gap between the rollers of the flaking system from 0.4 mm to 1.6 mm led to a decrease in the total output by 13.6%. The results of the research are given in Figure 4.

The complex dependence of the total product of the two systems can be explained by the fact that powdery products after the flaking mill fall into the inter-shale gap and the break system and create additional resistance to the destruction of large staircase particles of the endosperm. At the stage of flattening, particles of different sizes are formed. In the composition of these mixtures there are particles of flour, dunsts and grains that do not require grinding. Left in the total mass of the product, together with large staircase particles of the endosperm, they assume part of the efforts of the rollers and the break system, reducing the energy consumption that should go to the destruction of the endosperm, thereby reducing the potential technological efficiency of the grinding process (Kharchenko et al.,2015).

In the roller mill, the gap between the rollers was constant (0.5 mm), which means that with a decrease in the deformation of the grains during grinding in the roller mill, the yield of small products will increase. Based on this, the nature of the curve on Figure 4, can be explained by the fact that in the range of gaps between the rollers of the ivy machine from 0.4 to 0.8 mm, the yield of small products during flattening decreases much faster than their increase in the roller mill due to a decrease in deformation of grains. In the range of gaps between the rollers of the flaking mill from 0.8 to 1.6 mm, there is an intensive increase in the yield of small products in the roller mill much faster than their output decreases during flattening.

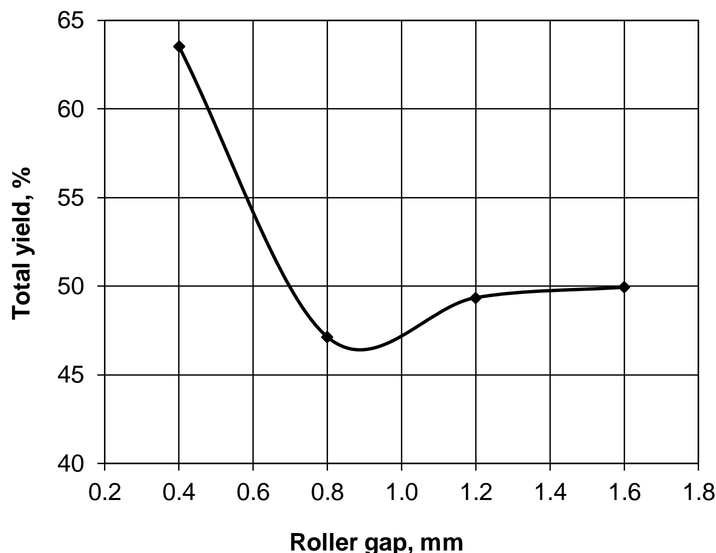

Figure 4. Total product of intermediate grinding products after flattening and grinding in the roller mill (in total)

In addition, the nature of the curve of the total product of the two systems was influenced by the size of the particles obtained after flattening, since the distance between the rollers of the roller mill remained unchanged, and the products of flattening with an increase in the distance between the rollers of the flaking mill increased their overall dimensions.

From both series of studies, the following conclusions can be drawn: if we take 1.4 mm as the optimal gap between the rollers of the flaking system, the total product of intermediate grinding products in the roller mill varies within 2.9% depending on the organization of the flattening and grinding process. Based on the results of research, it is impossible to give preference to the first or second method of grinding flattened wheat, since there are no significant differences between them with a gap value between the rollers of the flaking mill of 1.4 mm.

Analysis of granulometric characteristics of grinding products

To clarify the effect of the flattening process on the product of individual fractions of grinding products, granulometric characteristics of grinding products were investigated, provided that large products were crushed, and small products were previously isolated in the aspiration channel.

The differential curves in Figure 5 are predominantly the same. It is noteworthy that when grinding whole grain (control sample) in the roller mill, in the particle size range from 90 µm to 400 µm, the product of grinding products is greater than for flattened wheat grain. And from 400 µm to 1000 µm product of products is less than for products that have undergone flattening. For products that have passed flattening, regardless of the size of the gap between the rollers of the flaking mill, there is a smaller product of small fractions (less than 400 microns) and vice versa an increase in the product of large fractions.

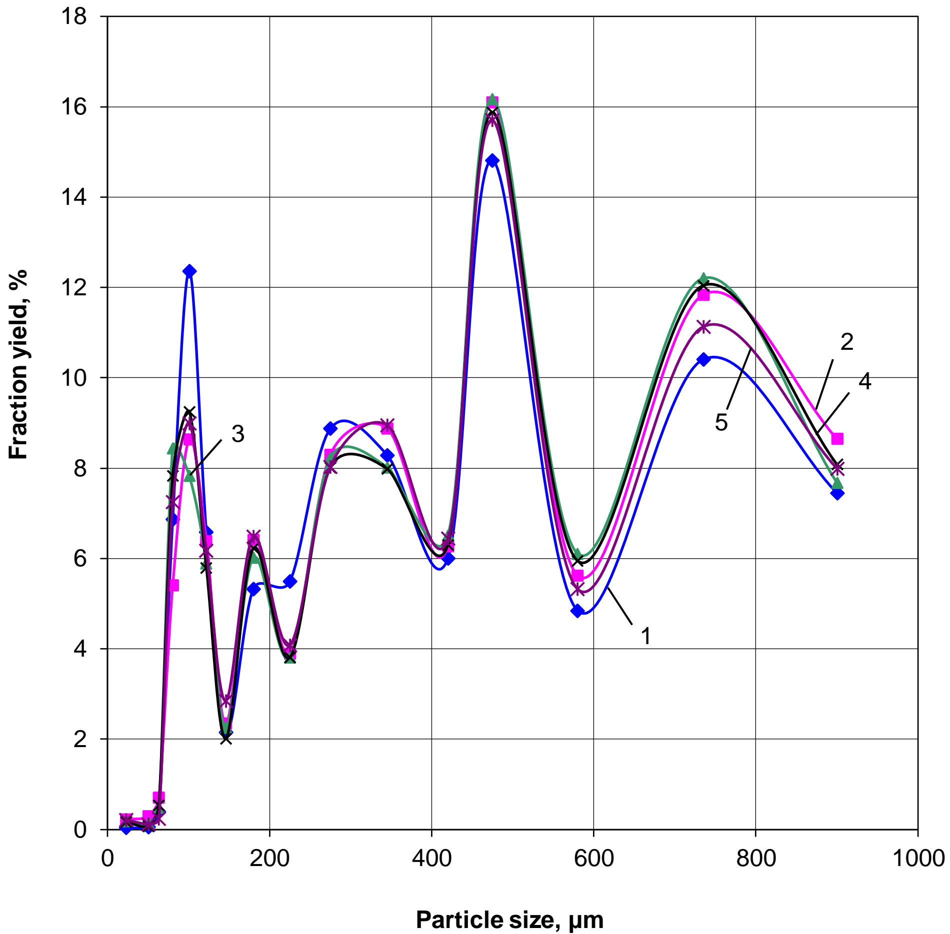

Figure 5. Differential distribution curves of intermediate grinding products after the roller mill (with selected small products after flattening):

1 – control sample (whole grain);
2 – value of the distance between the rollers of the flaking system is 0.4 mm;
3 – value of the distance between the rollers of the flaking system is 0.8 mm;
4 – value of the distance between the rollers of the flaking system is 1.2 mm;
5 – distance between the rollers of the flaking system is 1.6 mm.

From the results obtained, it can be concluded that large fractions are more crushed and moved to smaller ones when grinding whole grain under unchanged modes of operation of the roller mill.

Figure 5 shows that the output of individual fractions of intermediate grinding products has an uneven distribution in the total mass of the product, the differential curves are polymodal, as evidenced by the presence of five maximums in all curves. In fractions with the largest number of particles, the curve shows the maximum, and in the absence of particles,

the curve decreases to zero. The uneven distribution of particles in the mixture can be explained by the heterogeneity of the strength of the wheat endosperm, as well as the uneven efforts applied to particles during grinding (Chornyi et al., 2021).

Integral curves are less sensitive to changes in granulometric composition of product fractions than differential curves and are the preferred way to graphically analyze granulometric composition data (Chornyi et al., 2021).

The integral distribution curves of wheat grain grinding products under different conditions have an S-shaped view (Lyu et al., 2020) and are shown in Figure 6.

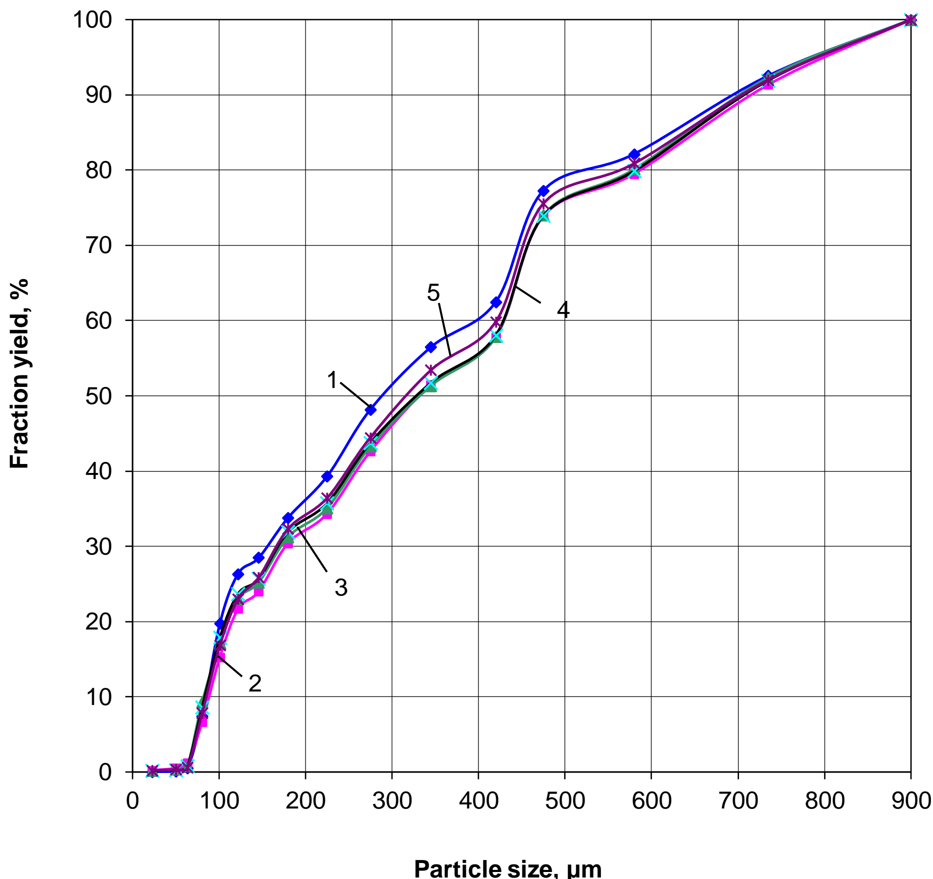

Figure 6. Integral particle distribution curves of intermediate grinding products after the roller mill (with small products isolated after flattening):
1 – control sample (whole grain);
2 – value of the distance between the rollers of the flaking system is 0.4 mm;
3 – value of the distance between the rollers of the flaking system is 0.8 mm;
4 – value of the distance between the rollers of the flaking system is 1.2 mm;
5 – distance between the rollers of the flaking system is 1.6 mm.

Analysis of integral curves showed that the largest yield of individual fractions of products was observed when grinding in the roller mill of the control sample, that is, the whole grain. Based on these integral curves, it can be concluded that in order to achieve a greater product of individual fractions of grinding products, it is necessary to adjust the distance between the rollers of the roller mill by increasing the total yield of grinding products.

The greater yield of individual fractions of products during grinding in the roller mill of the whole grain is explained by the fact that the wheat was not subjected to additional deformations and therefore, with a constant gap value between the rollers of the roller mill, greater grinding forces on individual grains were reflected, which gave a greater yield of individual fractions of products. Flattened grains, on the contrary, changed their overall dimensions and therefore, the rollers of the roller mill had less effect on the deformed grains, which led to a decrease in the yield of individual fractions of grinding products. The greater the gap between the rollers of the flaking mill, the greater the yield of fine fractions obtained in the roller mill. The obtained integral curves have a similar appearance to the integral curves obtained by Campbell G.M. and its collaborators (Campbell et al., 2007; Fang et al.,2003; Galindez-Najera et al., 2014; Galindez-Najera et al., 2016), which allows us to use the mathematical apparatus for their description, which was developed by G.M. Campbell.

Conclusions

Studies have confirmed the exponential dependence of the yield of intermediate products of flattening on the distance between the rollers of the flaking mill.

It is established that the modes of grinding of flattened grain in the roller mill and the shredded system depend on the mode of grain flattening in the flaking mill. The optimal distance between the rollers of the flaking mill is 1.4 mm. At this distance between the rollers of the flaking mill, the highest yield of intermediate products of grinding is observed in the roller mill of I shredded system.

The total yield of intermediate products after flattening and grinding in the roller mill I shredded system in the absence of intermediate separation of flattening products increases 2.9% more than under similar conditions for the separation of these products.

Particle size analysis showed that the differential curves are polymodal in nature with five maxima. When grinding rolled wheat, more large products are formed than when grinding whole wheat grain.

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Peculiarities of the lipid composition of sunflower wax

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Abstract

Introduction. The purpose of this study is to determine the peculiarities of the lipid composition of sunflower wax separated from crude and refined oil.

Materials and methods. Waxes were separated from triacylglycerols by silica gel column chromatography. Wax compositions were determined by gas-liquid chromatography with flame ionization detection. The column was thermostatted in a given mode at temperatures from 170 to 380 °C.

Results and discussion. The article presents experimental data for determining the composition of waxes in crude and refined sunflower oil. Gas-liquid chromatography conditions described in the study methodology expanded the range of detection of long-chain waxes. New data have been obtained about sunflower oils consisting of waxes containing 50 or more carbon atoms.

Waxes with hydrocarbon chain lengths of 44-56 carbon atoms (C44 to C56) have been identified in crude sunflower oil. In crude sunflower oil, waxes with chain lengths ranging from 46 to 52 carbon atoms (C46, C48, C50, and C52) account for up to 61% of the total waxes. Identified waxes with even numbers of carbon atoms accounted for 82% of the total waxes. Crude sunflower oil was found to have the highest content of wax with 48 carbon atoms (C48) of chain length.

Refined sunflower oil showed a decrease in the content of wax compounds by an order of magnitude. The refining process removed waxes with shorter C44 and C46 hydrocarbon chains. Refined sunflower oil contains trace amounts of waxes with chain lengths ranging from 48 to 54 carbon atoms (C48, C49, C50, C51, C52, C53, and C54). Moreover, the predominance of chains with even numbers of carbon atoms over those with odd numbers of carbon atoms in refined sunflower oil is emphasized. Refined sunflower oil is dominated by waxes with chain lengths of 50, 52 and 54 carbon atoms (C50, C52, and C54).

The acid and iodine values, the saponification number and the wax melting point have been analyzed, which are in line with an identified composition of sunflower wax.

Conclusion. The dependence of wax content on the chain lengths with even and odd numbers of carbon atoms both in crude and refined sunflower oil is parabolic.

Introduction

Given the ever-increasing production and consumption of sunflower oil year after year, studies of oil-related substances, such as waxes, and their composition is a matter of current interest (Oseyko et al., 2020; Oseyko et al., 2021). The applications of the separated wax are determined by its composition and properties. Sunflower wax is a natural thickener, structurant, and the wax derivatives can be used as emulsifiers, surface-active compounds, components of protective coatings of food and drugs, etc.

Wax is a natural product of a plant or animal origin and consists of compounds of different compositions, mainly from esters derived from higher carboxylic acids and higher alcohols (Oseyko[1] et al., 2019; Oseyko[1] et al., 2021). Wax is insoluble in water but readily soluble in organic solvents and hot alcohol. Substances whose chemical properties are close to the ones of waxes, but do not contain esters, are called waxy analogues or waxy compounds, such as paraffin. The composition of wax depends on the source and method of production (Romanovska, 2006). Beeswax is distinguished by the following extraction methods: melting, mechanical pressing, and chemical extraction. Soy wax separated from soybean oil during refining is promising (Oseyko, 2006).

Currently, there is growing interest in the lipid and blend composition of plant materials, namely the composition, quality, functional and health-improving and pharmacological properties of food products, additives and drugs (Oseyko et al., 2017; Oseyko et al., 2019; Farinon et al., 2020). Awareness of the properties of multicomponent lipid products of natural origin, in particular, waxes, substantiates their applications. The versatility of methods for obtaining waxes and controlling the composition and physicochemical parameters of lipid products gives an impetus to the search for optimal methods for obtaining waxes and the study of their physicochemical parameters.

Among other sources of wax are lipid-containing materials, such as used filter powder after filtering frozen oil (Omelchenko et al., 2016), tank sediments self-precipitated during oil storage, and sunflower husks (Kleiman et al., 1969). Sunflower wax extraction methods are labor-intensive, resource-intensive, and time-consuming. These factors explain the currently insufficient information on the composition of sunflower wax lipids, and at the same time, its limited use in various fields of human activity. A review of the literature revealed an understudied composition of sunflower wax.

The purpose of this study is to determine the peculiarities of the lipid composition of sunflower wax separated from crude and refined oil.

Materials and methods

The study materials are crude sunflower oil and sunflower oil refined by classical refining process (Oseyko, 2006) (neutralization, freezing, bleaching, deodorization), as well as sunflower wax obtained by freezing and filtering from the used perlite powder. Samples of oils and used filter powder were obtained from the Kyiv Margarine Factory “Olkom”.

Methodology for the preparation of test samples

Before the chromatographic analysis of waxes, oil samples were passed through a silica gel column to purify the wax from related substances (Patent UA114235C2, Sposib khromatohrafichnoho vyznachennia voskopodibnykh rechovyn, M.I. Oseiko, I.V. Levchuk, V.A. Kishchenko, T.I. Romanovska, 2017).

The wax was eluted with a mixture of n-hexane/chloroform in a ratio of 7:3 (Patent UA114235C2, 2017).

The resulting eluate was boiled down to dry residue under vacuum at 60 °C and dissolved in 1 cm^3 of chloroform (Patent UA114235C2, 2017).

2.0 x 10^{-3} cm^3 (2 µl) of the prepared wax sample was added to the GC column (Gordon, 1990).

Study methodology

Wax composition analysis

Wax compositions were determined by gas-liquid chromatography with flame ionization detection (Gordon, 1990). Wax content of oils is determined by gas-liquid chromatography at the capillary column's maximum thermostating temperature of 320 – 380 °C (Kishchenko, 2014; Levchuk et al., 2016).

Wax separation

Wax separation was carried out in a silica column with a diameter of 0.32 mm and a length of 15 m filled with a chemically inert liquid stationary phase (5% diphenyl 95% dimethyl polysiloxane). The stationary phase does not volatilize at column operating temperatures, has a low viscosity, and forms a continuous, uniform film. Nitrogen flowing at a rate of 20 cm^3 per minute served as the mobile phase that moved the wax sample through the column. The samples were passed through a GC column at temperatures starting from 170 °C and holding time of 1 minute with a heating rate of 6 °C per minute up to 380 °C and holding time of 20 minutes. The evaporator temperature was maintained at about 370 °C and that of the detector at 400 °C. The flame ionization detector was operated at the hydrogen flow rate of 30 cm^3/min and air flow rate of 350 cm^3/min. (Gordon, 1990).

Processing of results

The composition and content of waxes in the test samples were determined with three repeated measurements using the Agilent Technologies Chemstation software (Gordon, 1990; https://www.agilent.com).

Removal of frozen sunflower wax

The wax separated during freezing with an auxiliary filter material (filter perlite powder) was heated in a wax melter with a woven sieve in a cabinet dryer at a temperature of 80 °C within 3 hours until the wax was completely melted. The resulting filtered wax was crystallized at room temperature and analyzed.

Determination of Physicochemical Parameters of Sunflower Wax

For sunflower wax extracted by freezing, free fatty acid content (acid value), hydrolysable ester content (saponification value), unsaturated hydrocarbon chain content (iodine value) were determined by conventional titrimetric methods, and the wax melting point was determined using an open capillary tube (Mank et al., 2018).

Results and discussion

Lipid composition of sunflower wax

The study of the composition of waxes has some difficulties associated with the breaking of the separated multicomponent wax fraction into constituent elements in a thin layer of a capillary column and their detection using a detector. The composition of waxes is determined with a flame ionization detector and tracking substances (separated and purified substances) or mass spectrometry with a subsequent search in a mass spectral database of the compounds that may be present in test samples (Gordon, 1990). According to their properties, waxes belong to substances with a melting point of up to 80 °C (Gunstone, 2002). During the introduction of the sample, the temperature of the evaporator is maintained at about 370 °C and that of the flame ionization detector at about 400 °C so that the wax sample is transferred and distributed in the mobile gaseous phase (Gordon, 1990). The use of high separation and detection temperatures is due to the physical properties of the test substances.

The prepared sunflower wax samples were analyzed by gas-liquid chromatography using a flame ionization detector. Tables 1 and 2 present experimental data for determining the composition of waxes in sunflower oil.

Table 1

Composition of crude and refined sunflower oil wax, mg/g (n=3, P≥0.95)

Name of oil sample	C44	C46	C48	C50	C52	C54	C56	Other*	Total
Crude	0.028	0.046	0.059	0.055	0.050	0.036	0.011	0.064	0.349
Refined	0.001	0.001	0.002	0.003	0.003	0.003	0.001	0.006	0.020

Other* – hydrocarbon chains with an odd number of carbon atoms C43, C45, C47, C51, and C53 combined

Table 2

Composition of crude and refined sunflower oil wax with an odd number of carbon atoms, mg/g (n=3, P≥0.95)

Name of oil sample	C43	C45	C47	C49	C51	C53
Crude	0.007	0.007	0.009	0.012	0.015	0.014
Refined	-	0.001	-	0.002	0.002	0.001

Tables 1 and 2 show that oil samples mainly contain waxes with even numbers of carbon atoms in the hydrocarbon chains. Waxes with hydrocarbon chain lengths of 44-56 carbon atoms (C44 to C56) have been identified in crude sunflower oil. In crude sunflower oil, waxes with chain lengths ranging from 46 to 52 carbon atoms (C46, C48, C50, and C52) account for up to 61% of the total waxes. Refined oil is mainly characterized by hydrocarbon chains with even numbers of carbon atoms (C48, C50, C52, and C54), with waxes containing odd numbers of carbon atoms constituting the minority.

Refined sunflower oil showed a decrease in the content of long-chain wax compounds by an order of magnitude. The refining process probably removes waxes with shorter C44 and C46 hydrocarbon chains. Refined sunflower oil mainly contains waxes with chain

lengths ranging from 48 to 54 carbon atoms (C48, C49, C50, C51, C52, C53, and C54). That said, the predominance of chains with even numbers of carbon atoms over those with odd numbers of carbon atoms in refined sunflower oil is outspoken.

The thermostating temperature of the capillary gas-liquid chromatographic column of 380 °C and the detection mode enable the detection of waxes with hydrocarbon chain lengths of up to 56 carbon atoms (C56). Most waxes have been found to contain even numbers of carbon atoms, with the even-carbon-numbered waxes in the test sample accounting for 82% of the total number of waxes.

The conditions for the preparation of a sample for analysis and the gas-liquid chromatography temperature conditions described in the study methodology expanded the range of detection of long-chain waxes. Compared to studies (Carelli et al., 2002), we obtained new findings on the presence of waxes with hydrocarbon chain lengths of 50 carbon atoms and more in sunflower oil.

Tables 1 and 2 show that the dependence of wax content on the chain lengths with even and odd numbers of carbon atoms both in crude and refined sunflower oil is parabolic. Crude sunflower oil was found to have the highest content of wax with 48 carbon atoms (C48) of chain length. Refined sunflower oil is dominated by waxes with chain lengths of 50, 52 and 54 carbon atoms (C50, C52, and C54).

In a study (Carelli et al., 2002; Chalapud et al., 2016), oil waxes were preliminarily separated using wet silica gel with 2% moisture content. A mixture of n-hexane/diethyl ether in a ratio of 8.5:1.5 served as the wax eluent. Sudan dye is added to the column together with the oil in order to determine whether the wax fraction has been eluted from the silica gel since waxes are separated in the first place, followed by the dye, and finally triacylglycerols. The wax was separated using a gas-liquid chromatograph with a capillary column (11 m long, 0.32 mm in diameter) and 0.52 μm thick film of the liquid stationary phase containing 5% diphenyl and 95% dimethylpolysiloxane. The temperature in the column was increased from 80 to 200 °C at a rate of 30 °C/min, maintained at 200 °C for 1 minute, and then increased to 340 °C at a rate of 3 °C/min. The composition of the separated wax fraction was determined with a flame ionization detector at a temperature of 350 °C. The composition of waxes in crude oil containing 0.995 mg/g of waxes is given in Table 3.

Table 3

Chromatographically determined composition of waxes in crude sunflower oil (Carelli et al., 2002), in % of total wax content

Sample	C36	C38	C40	C42	C44	C46	C48	C37	C39	C41	C43
Oil	13.0	4.2	11.5	7.5	7.0	11.2	13.9	9.2	3.4	14.3	4.8

Comparison of the data in tables 1 and 2 with the data in table 3 (Carelli et al., 2002) showed a significant effect of the temperature conditions of the chromatographic separation on the identification of waxes. Our data are related to the data (Carelli et al., 2002) in terms of the content of waxes with carbon atom numbers ranging from 44 to 48 (C44, C46, and C48). We believe that our inability to detect waxes containing more than 50 carbon atoms was due to the temperature in the chromatographic column maintained at 340 °C. The identified wax composition (Carelli et al., 2002) is incomplete as regards the presence of long-chain waxes with even and odd carbon numbers.

The wax fraction separated in a silica gel column was subjected to saponification (Carelli et al., 2002). The saponified sample was further extracted with diethyl ether to obtain

a hydroalcoholic fraction with fatty acids and a diethyl ether fraction with fatty alcohols. Following the conversion of fatty acids to methyl esters and fatty alcohols to trimethylsilyl ethers, they were subjected to the gas-liquid chromatographic analysis. The composition of fatty acids and fatty alcohols in the wax fraction is given in Table 4.

Table 4

Composition of fatty acids and fatty alcohols in the wax fraction of crude sunflower oil (Carelli et al., 2002), wt% of the total amount

Fatty acids	wt%	Fatty alcohols	wt%
C14:0	1.4	C16	4.3
C16:0	9.8	C18	23.1
C16:1	1.2	C19	18.4
C18:0	4.9	C20	2.0
C18:1	18.6	C22	7.5
C18:2	44.0	C23	0.6
C18:3	1.3	C24	11.9
C20:0	4.3	C25	1.8
C20:1	1.0	C26	9.3
C21:0	0.2	C27	0.5
C22:0	9.7	C28	8.3
C22:1	0.7	C29	0.6
C24:0	0.1	C30	7.9
C26:0	0.9	C32	3.8
C27:0	0.2		
C28:0	1.0		
C29:0	0.3		
C30:0	0.4		

Table 4 shows that the wax fraction of sunflower oil mainly contains the following fatty acids: linoleic (C18:2), oleic (C18:1), palmitic (C16:0), behenic (C22:0). Among fatty alcohols, the wax fraction mainly contains octadecanol (C18), nonadecanol (C19), and tetracosanol (C24).

Sunflower oil waxes were subjected (Reiter et al., 2001; Broughton et al., 2018) to the gas-liquid chromatographic analysis using a flame ionization detector together with mass spectrometry of three different oil samples. The results were presented as a percentage of the total wax content. The content of C42:0 wax ester determined using a flame ionization detector in the range of 6.2-6.9% correlates to the content determined using mass spectrometry (6.1%). The content of C44:0 wax ester determined with a flame ionization detector is in the range of 3.8-12.0%, and the content determined using mass spectrometry is 1.9%. The content of C46:0 and C46:1 wax esters determined with a flame ionization detector was 4.1 and 5.7%, respectively, and the content determined using mass spectrometry was 3.5%. Among other wax esters were phytol esters with one of the fatty acids C18:1, C18:0, C20:0, C22:0, C24:0, and geranylgeranyl esters with one of the listed fatty acids C18:1, C18:0, C20:1, C20:0, C22:0, C24:0 (Reiter et al., 2001). The inability to detect long-chain waxes in oil was probably due to the fact that the maximum temperature of the capillary column during gas-liquid separation did not exceed 350 °C.

Tank sediments and sunflower seed shells (husks) serve as significant reserve sources of sunflower wax extraction. The wax content of tank sediments of sunflower oil and oil from sunflower husks was determined (Redondas et al., 2020; Kleiman et al., 1969). The wax composition was identified by gas-liquid chromatography in the temperature range of 100-400 °C using a flame ionization detector. Data on the composition of the detected waxes in the tank sediments of sunflower oil and oil from sunflower husks are given in Table 5.

Table 5

Composition of waxes in the tank sediments of sunflower oil and oil from sunflower husks (Kleiman et al., 1969), in % of the total wax content

Wax carbon chain length	Wax content of tank sediments	Wax content of oil from sunflower husks
C42	4.0	5.1
C43	0.8	-
C44	21.2	16.0
C45	2.3	1.3
C46	23.9	19.4
C47	1.9	1.4
C48	15.4	15.5
C49	1.7	1.8
C50	8.4	12.6
C51	0.2	0.9
C52	7.7	10.0
C53	0.7	traces
C54	4.8	7.4
C55	0.6	-
C56	3.7	4.4
C57	-	-
C58	1.4	2.1
C59	0.5	-
C60	0.7	1.2
C61	-	0.7
C62	-	0.2

The composition of the wax determined in sunflower oil according to tables 1 and 3 and in sunflower wax from tank sediments according to table 5 shows a general trend in terms of the content of substances with a chain length of 44, 46, 48 carbon atoms (C44, C46, C48). However, sunflower oil has the highest content of wax with C48 carbon atoms, while tank sediment mainly contains wax with C46 carbon atoms. We proceed from the assumption that this is due to the oil storage conditions and duration, the conditions of formation of tank sediments and the polydispersity of the system, which, in addition to acylglycerols and waxes, has a significant amount of condensed moisture and phospholipids exhibiting surface activity. In this kind of system, even and odd carbon numbered wax esters containing C50 and more carbon atoms are more stable due to intermolecular interactions with triacylglycerols and related oil substances.

Sunflower oil tank sediments are predominantly composed of waxes with even carbon numbered hydrocarbon chains, namely C44, C46, C48, C50, C52, C54, and C56, which combined account for 76.6% of the total number of waxes identified.

Physicochemical parameters of sunflower wax samples

The wax sample separated from perlite filter powder was white, malleable and non-brittle. After melting and separating from perlite, the wax sample had a uniform matte straw color, was oily to the touch, and had a uniform and malleable structure. Physicochemical parameters of the wax are given in Table 6.

Table 6

Physicochemical parameters of the sunflower wax

Indicator	Wax sample	Hexane-extracted wax (Omelchenko et al., 2016)
Acid value, mg KOH/g	12.2 ± 0.6	7
Saponification value, mg KOH/g	111.5 ± 1.5	109
Iodine value, mg KOH/g	10.8 ± 0.4	8
Melting point, °C	69.0 ± 0.5	81

Physicochemical parameters in table 6, namely the iodine value, indicate that the wax esters of the wax sample mainly contain saturated acyls. The data are consistent with the data in table 3 (Carelli et al., 2002) and findings (Omelchenko et al., 2016). Table 4 (Carelli et al., 2002) shows that the crude sunflower oil wax, in addition to saturated fatty acids, has been found to contain unsaturated fatty acids, such as palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), gadoleic (C20:1), and erucic (C20:1). Our data (Table 6) correspond to the data (Table 4) in terms of the wax iodine value, i.e. the degree of unsaturation of the fatty acid radicals of the wax compounds.

Sunflower wax contains ester bonds that undergo hydrolysis under the action of alkalis or acids, as indicated by the saponification value and acid value. The acid value indicates an acceptable free fatty acid content.

The melting point of the wax sample was below the melting point of the hexane-extracted wax. We assume that this fact can be explained by the duration of the processes of wax extraction from the used filter powder. Extraction is carried out using the exhaustive extraction technique by Soxhlet (Mank et al., 2018) and the whole process takes about 24 hours. Extraction of the wax sample by melting, filtering and crystallization took 6 hours. The shorter heating time of the material and the fatty acid profile of the wax esters may have resulted in a lower melting temperature of the wax sample.

Conclusion

The peculiarities of the lipid composition of sunflower wax separated from crude and refined oil have been determined.

1. In crude sunflower oil, waxes with chain lengths ranging from 46 to 52 carbon atoms (C46, C48, C50, and C52) account for up to 61% of the total waxes. Identified waxes with even numbers of carbon atoms accounted for 82% of the total waxes.

2. Refined sunflower oil showed a decrease in the content of wax compounds by an order of magnitude. The refining process removed waxes with shorter C44 and C46 hydrocarbon chains. The refined oil has been found to contain trace amounts of waxes.
3. The dependence of wax content on the chain lengths with even and odd numbers of carbon atoms both in crude and refined sunflower oil is parabolic. Crude sunflower oil was found to have the highest content of wax with 48 carbon atoms (C48) of chain length. Refined sunflower oil is dominated by waxes with chain lengths of 50, 52 and 54 carbon atoms (C50, C52, and C54).
4. The acid and iodine values, the saponification number and the wax melting point have been analyzed, which are in line with an identified composition of sunflower wax.

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Bioactive compounds and antioxidant activity of beetroots prepared by different drying methods

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Abstract

Introduction. The aim of the research is to investigate the influence of different drying methods, including freeze drying, vacuum drying, microwave drying and microwave vacuum drying, on the bioactive compounds and antioxidant activity of beetroots.

Materials and methods. Bioactive compounds and antioxidant activity of dried beetroots prepared by freeze drying (FD), vacuum drying (VD), microwave drying (MD), and microwave vacuum drying (MVD) were investigated. Moisture analyzer was used to determine moisture content of beetroots. Beetroot powder was extracted three times with 50% ethanol (v/v). Colorimetric methods were used to determinate contents of betalain, ascorbic acid and total phenolic, and 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of dried beetroots.

Results and discussion. Drying methods significantly affected the drying time, bioactive compounds and antioxidant activity of beetroots. The shortest drying time (0.56±0.01 h) was occurred at MVD, while FD required the longest drying time of 29.67±0.58 h. There was no significant difference in the final moisture content of dried beetroots prepared by different drying methods. It was found that the dried beetroots prepared by VD showed the highest ascorbic acid content of 8.73±0.23 mg/g, and the beetroots dried by FD displayed the highest betaxanthin content of 2.57±0.01 mg/g, while the beetroots obtained by MD showed the lowest betacyanin content of 2.98±0.02 mg/g, and FD led to significantly lower total phenolic content of beetroots in comparison with other drying methods. In terms of antioxidant activity, the beetroots prepared by MD and MVD showed significantly higher DPPH radical scavenging activity than the beetroots prepared by VD and FD. Meanwhile, the beetroots obtained by VD exhibited the largest ABTS radical scavenging activity of 17.22±0.35 mg trolox equivalent (TE)/g. Besides, the beetroots prepared by MVD displayed the highest FRAP value of 13.36±0.17 mg TE/g. Compared to beetroots prepared by other drying methods, the beetroots prepared by FD showed the lowest FRAP, DPPH and ABTS values, indicating the weakest antioxidant activity.

Conclusions. Considering the drying time, bioactive compounds and antioxidant activity, MVD is recommended to prepare dehydrated beetroots, which can reduce drying time and better preserve bioactive compounds and antioxidant activity of beetroots.

Introduction

Beetroot (*Beta vulgaris* L.) is an important root vegetable and widely grown in Russia, Germany, Ukraine, Turkey, Poland, France, Egypt, America, United Kingdom and China. According to the macronutrient data reported by the US Department of Agriculture, 100 g of raw beetroot contains an average energy of 43 kcal, total sugar 6.76 g, total dietary fiber 2.8 g, proteins 1.61 g, and total lipids 0.17 g (United States Department of Agriculture, 2013). Beetroot can not only be consumed as fresh vegetable, but also can be processed into canned, pickled, frozen and dehydrated products (Kanner et al., 2001; Paciulli et al., 2016). Beetroot contains highly bioactive compounds, such as betalains (betacyanins and betaxanthins), polyphenols, flavonoids, carotenoids, ascorbic acid, nitrate and saponins, Antioxidant activity of bioactive compounds and successful utilization of beetroot in disease prevention and health promotion is increased in last few decades (Chhikara et al., 2019). Beetroot spoils rapidly due to high moisture content, continuous metabolism and microbial attack.

Drying is one of most widely used methods for preservation foods by inhibiting enzyme activity, avoiding microbiological spoilage, and delaying deterioration (Arikan et al., 2012). In order to obtain dried vegetables, a great number of drying methods have been applied such as hot air drying, vacuum drying, microwave drying, microwave vacuum drying, and freeze drying. During drying process, it causes nutrient losses and changes the raw material's chemical and physical properties. Thus, choosing the most suitable drying method mainly depends on the quality of the final products (Wei et al., 2019). Vacuum drying (VD) is a very good drying technology because of its sealed environment, low content of oxygen and cost superiority (Dai et al., 2021). A number of studies have demonstrated that VD can well preserve the nutritional components, physical and chemical properties, organizational structure and sensory quality of products (Mitra et al., 2011; Liu et al., 2021). Microwave drying (MD) is also widely used in the drying of agricultural products, it is generally recognized as rapid drying rate, short drying time, rapid process control, and fast-switching (Jin et al., 2017). At the same time, MD causes non-uniform heating, textural damage, nutrient degradation, and limited penetration depth (Vadivambal et al., 2010). Microwave vacuum drying (MVD) is an alternative drying method for a variety of foodstuffs. The intensive heating of microwave and low boiling point generated by the vacuum make the food to be dried at relatively low temperature and in a short time, helping to maintain a high level of healthy nutrition and sensory quality (Scaman et al., 2005). As the best dehydration method for heat sensible foods, freeze drying (FD) involves high energy consumption, high equipment costs, and long drying time, which limits its use in the production of high-value products (Köprüalan et al., 2021).

The drying method significantly influences the bioactive compounds and antioxidant activity of the final product. Therefore, it is important to choose a suitable drying method to obtain high quality of dried products. In literature, there are rarely studies on the effects of different drying methods on the quality attributes of dried beetroots. So we conducted different types of drying methods to dry beetroots, exploring the effect of different drying methods on the quality of dried beetroots, and looking forward to obtaining high-quality dried beetroots in this study. Therefore, the purpose of the research is to investigate the influence of four different drying methods, including freeze drying, vacuum drying, microwave drying and microwave vacuum drying, on the bioactive compounds and antioxidant activity of dried beetroots.

Objectives of research:

- To explore the drying time and final moisture content of dried beetroots under four different drying methods.

- To investigate the effect of four different drying methods on the bioactive compounds of beetroots.
- To evaluate determine the antioxidant activity of dried beetroots obtained by four different drying methods.

Materials and methods

Materials

Fresh beetroots were purchased from a local market in Xuzhou, Jiangsu province, China. The fresh beetroots were washed, peeled, and cut into slices 75 mm in diameter and 4 mm in thickness. Ascorbic acid assay kit was obtained from Nanjing Jiancheng Institute of Bioengineering, Nanjing, China. Folin-Ciocalteu's phenol reagent (1.0 mol/L), gallic acid (purity≥98%), tri-2-pyridyl-s-triazine (TPTZ, purity≥98.0%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox,purity≥98.0%), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, purity≥98.0%) and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, purity≥98.0%) were obtained from Shanghai Yuanye Bio-Technology Co., Ltd, Shanghai, China.

Drying procedures

The experimental procedures of four different drying methods are listed as follows:

Microwave vacuum drying (MVD): The 300-g fresh beetroot slices were placed uniformly on a tray (61 cm × 43 cm × 5 cm), and the tray was put into a microwave vacuum dryer (WBZ-10, Guiyang Xinqi Microwave Industry Co., Ltd, Guiyang, China). The vacuum degree was –90 KPa. Microwave power of 1000W was investigated in microwave vacuum drying for beetroots at constant vacuum degree.

Microwave drying (MD): The 300-g fresh beetroot slices were spread evenly as a thin layer on the circular fiberglass tray (diameter-30 cm) of microwave drying system (SAM-255, CEM Corporation, USA), and dried at 390W.

Vacuum drying (VD): The 300-g fresh beetroot slices were spread a thin layer on a tray (31 cm × 29 cm × 2 cm) of vacuum drying oven (BPZ-6033B, Shanghai Yiheng Scientific Instrument Co., Ltd, Shanghai, China). The vacuum degree was –95 KPa. The temperature of vacuum drying was set at 60 °C.

Freeze drying (FD): The 300-g fresh beetroots were frozen at –20 °C for 12 h and were placed on the cavity in the freeze dryer (FDU-2110, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) at 4 Pa. The condenser temperature was set at –80 °C.

All the drying process stopped when the final moisture content of beetroots was less than 6.0% on a wet basis. All the drying experiments were conducted in three replications.

Determination of moisture content

The moisture content (wet basis) of beetroots was measured by a moisture analyzer (HX204, Mettler Toledo Co. Ltd., Switzerland) at 105 °C until it reached to a constant weight. The average initial moisture content of fresh beetroots was 90.07±0.72% (wet basis).

Extraction process

Dried beetroots obtained from triplicate were mixed and then ground into powders (pass through a 60-mesh sieve) to obtain samples with representative chemical components for particular drying methods. 2.0 g of beetroot powder was placed in a 50-mL centrifuge tube, 20 mL of 50% ethanol (v/v) was added, and then fully mixed by a vortex mixer (VORTEX-5 Kylin-Bell Instrument Manufacturing Co., Ltd, Jiangsu, China) for 2 min. After centrifugation using a centrifuge (H1850, Xiangyi Centrifuge Instrument Co., Ltd, Hunan, China) at 5000 rpm for 10 min, the supernatant was collected, and the precipitate was extracted twice with 20 mL of 50% ethanol (v/v). The supernatants obtained from three extractions were combined and diluted to 100 mL with 50% ethanol (v/v). The extracts were stored at 4 °C until further analysis.

Determination of bioactive compounds

Betalains are natural water-soluble pigments containing nitrogen, which are subdivided into betacyanins (red-violet pigments) and betaxanthins (yellow-orange pigments) (Coy-Barrera, 2020). The betalain content was determined spectrophotometrically (722N, Shanghai Youke Instrument Co., Ltd, Shanghai, China) as described by Stintzing et al., 2005. The extract was diluted with 0.05 mol/L phosphate buffer solutions (pH 6.5) to obtain absorption values of $0.8 \leq A \leq 1.0$ at 538 nm, and then the absorbance of diluted extract was measured at 480 nm, 538 nm, and 600 nm, respectively. The betalain content was expressed as micrograms per gram (mg/g).

Ascorbic acid content of beetroots was determined by colorimetric method using a detection kit (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China). Results were calculated on a dry weight basis and expressed as mg/g.

Total phenolic determination was adopted from Folin-Ciocalteu method (Alvarez-Parrilla et al., 2011). Diluted extract (0.5 mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu's reagent (v/v), and then 2 mL of 7.5% sodium carbonate (w/v) was added. The mixture was incubated for 15 min at 50 °C and then cooled to room temperature, and the absorbance was measured at 760 nm by a visible spectrophotometer (722, Shanghai Youke Instrument Co., Ltd, Shanghai, China). A stand curve was obtained using different concentrations (0–0.1 mg/mL) of gallic acid. The total phenolic content of dried beetroots was expressed as milligrams of gallic acid equivalent (GAE) per gram.

Determination of antioxidant activity

Antioxidant activity of dried beetroots was evaluated by three methods.

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay was analyzed according to the colorimetric method with slight alterations (Brand-Williams et al., 1995). The DPPH assay was performed by adding 2 mL of diluted extract to 4 mL of 0.2 mM DPPH solution and reacting for 30 min in the dark at room temperature. The absorbance of the mixture was read at 517 nm. A calibration curve was obtained using different concentrations (0–200 μmol/L) of trolox. Results were expressed as milligrams trolox equivalent (TE) per gram.

The 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay was evaluated according to the method reported by Re et al., 1999, with minor modifications. Equal quantities of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ solution were mixed to obtain $ABTS^{\bullet+}$ solution. The $ABTS^{\bullet+}$ solution was allowed to stand for 16 h in the dark at room temperature. The $ABTS^{\bullet+}$ solution was diluted with 80% ethanol (v/v) to obtain an absorbance of

0.70±0.02 at 734 nm before the measurement. Diluted extract (0.4 mL) was reacted with 3.6 mL diluted $ABTS^{\bullet+}$ solution for 6 min at room temperature. Absorbance was recorded at 734 nm. Trolox with a concentration of 0–150 μmol/L was used as the standard curve. The results were expressed as trolox equivalent (TE) in mg/g.

The ferric reducing antioxidant power (FRAP) assay was conducted by the colorimetric method described by Benzie et al., 1996. First of all, FRAP reagent was prepared by mixing 0.01 M Tri-2-pyridyl-s-triazine (TPTZ) solution (prepared in 0.04 M HCl), 0.02 M $FeCl_3$ solution and 0.3 M acetate buffer (pH 3.6) at the volumetric ratio of 1:1:10. The diluted extract (0.2 mL) was fully reacted with 6 mL of FRAP reagent. After incubating at 37 °C for 10 min, the absorbance at 593 nm was read. A calibration curve was obtained using different concentrations (0–600 μmol/L) of trolox. All solutions were prepared on the day of use. Results were calculated as mg TE/g.

Statistical analysis

All experiments were conducted at least in triplicate and results were expressed as mean±standard deviation (SD). The differences among samples were determined by one way analysis of variance (ANOVA) followed by the Tukey's multiple range test using the SPSS Statistics Version 20 (IBM Corporation, Chicago, IL, USA). The mean values with different letters are significantly different at 95% confidence level ($p<0.05$). Origin 2017 (Origin Lab, MA, USA) was used to draw figures.

Results and discussion

Drying time and moisture content

The drying time and final moisture content of beetroots are depicted in Table 1. Different drying methods resulted in significant differences in drying time. FD had the longest drying time (29.67±0.58 h). Although FD has less thermal damage to products, it takes longer time. It is an expensive process and is only applicable to high-value products (Hu et al., 2006). MVD was much faster drying method than VD and FD. It required the shortest drying time (0.56±0.01 h) for MVD to reach the final moisture content. The drying time of MVD was reduced by 98.1% compared to FD, and was only 7.2% of the vacuum drying time. While the drying time for MD was 0.77±0.03 h and very close to MVD. The drying time required from MD reduced up to 90.2% in comparison with VD, and was about 2.6% of FD time. This indicated that the drying time was significantly reduced as microwave was used.

Table 1

Drying time and final moisture content of beetroots

Drying methods	MVD	MD	VD	FD
Drying time, h	$0.56±0.01^{c}$	$0.77±0.03^{c}$	$7.83±0.29^{b}$	$29.67±0.58^{a}$
Final moisture content, %	$5.47±0.71^{a}$	$4.82±0.60^{a}$	$4.41±0.81^{a}$	$4.36±0.70^{a}$

Note: Different superscript letters in the same row indicate a significant difference ($p<0.05$). MVD, microwave vacuum drying; MD, microwave drying; VD, vacuum drying; FD, freeze drying.

Moisture content is essential for the quality control and stability of dried foods. Lower moisture content usually represents longer shelf life. As presented in Table 1, the final moisture content of beetroots obtained by four different dying methods ranged from 4.36±0.70% to 5.47±0.71%. VD and FD beetroots had slightly lower final moisture content comparing to MVD and MD, and there was no significant difference in final moisture content among all the beetroots prepared by different drying methods.

Effect of different drying methods on the bioactive compounds of beetroots

The influence of different drying methods on betalain content of dried beetroots is displayed in Figure 1. As can be seen, the betacyanin content of dried beetroots obtained by four different drying methods ranged from 2.98±0.02 to 4.04±0.05 mg/g. It was found that the beetroots obtained by MD showed the lowest betacyanin content (2.98±0.02 mg/g). The greatest betacyanin content (4.04±0.05 mg/g) of dried beetroots was observed in VD, and the beetroots prepared by VD showed no significant difference from that prepared by FD in betacyanin content (p>0.05). As it can be seen from Figure 1, the betaxanthin content of dried beetroots prepared by four different drying methods ranged from 2.40±0.02 to 2.57±0.01 mg/g, and the greatest betaxanthin content (2.57±0.01 mg/g) of dried beetroots was obtained from FD. No significant difference was observed in MVD and MD (p>0.05).

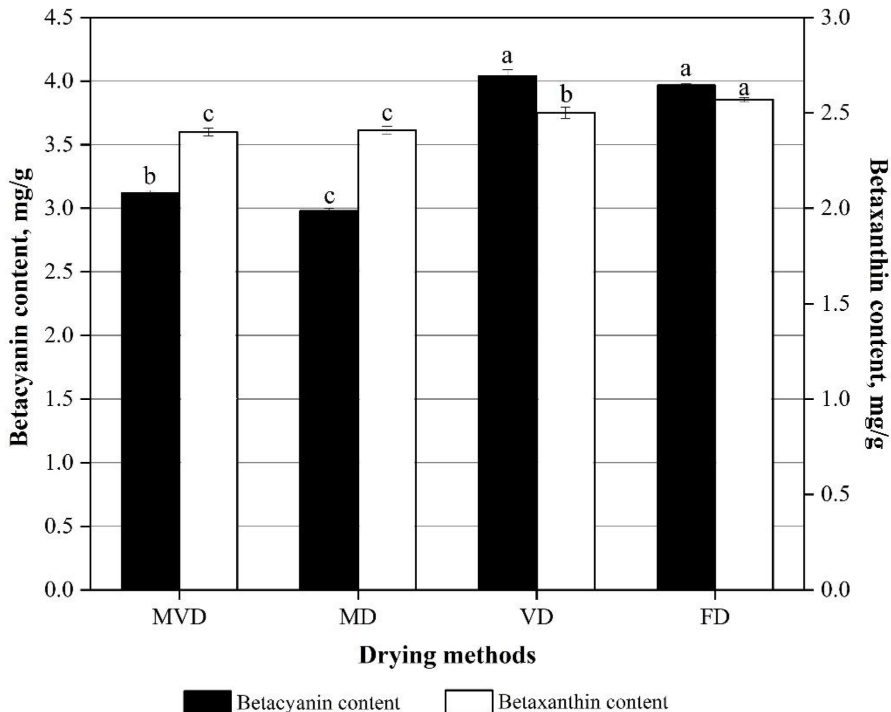

Figure 1. Betalain content of dried beetroots as affected by different drying methods. MVD, microwave vacuum drying; MD, microwave drying; VD, vacuum drying; FD, freeze drying. Means with different letters in each column indicate significantly different (p<0.05).

Ascorbic acid is relatively unstable to heat, oxygen, and light, and easily is degraded during drying process, so it can be used as an indicator for the quality of dried products (Hu et al., 2006). The higher the temperature, the longer the drying time, and the more degraded ascorbic acid in dried fruits and vegetables (Cui et al., 2004). The effect of different drying methods on the ascorbic acid content is provided in Figure 2. The results showed that the beetroots prepared by VD displayed the highest ascorbic acid content of 8.73±0.23 mg/g, followed by MD, FD, and MVD beetroots of 8.32±0.10, 7.44±0.06, and 7.17±0.09 mg/g, respectively. MVD and FD beetroots had relatively lower ascorbic acid content than that of MD and VD beetroots, and there was no significant difference between MVD and FD (p>0.05). This can be explained that microwave vacuum drying caused some higher temperature areas on the surface of the beetroots, resulting in loss of the heat-sensitive ascorbic acid (Wei et al., 2019).

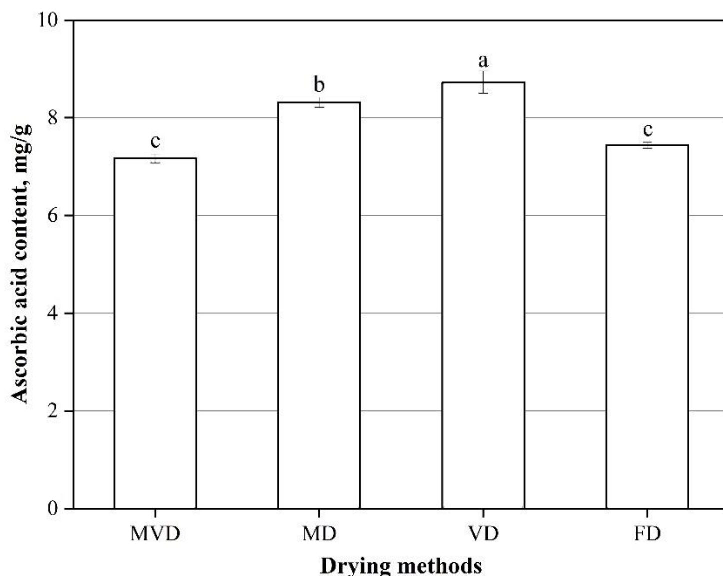

Figure 2. Ascorbic acid content of dried beetroots as affected by different drying

Figure 3 shows the total phenolic content of dried beetroots obtained by different drying methods. The highest total phenolic content was observed in VD (10.23±0.08 mg GAE/g) beetroots, followed by MVD (10.20±0.08 mg GAE/g), MD (9.31±0.14 mg GAE/g), whereas FD (8.01±0.11 mg GAE/g) beetroots showed the lowest content. Similar results have been reported by other researchers. It was reported that freeze drying of willow led to a lower total phenolic content than thermal drying methods such as hot air drying, oven drying and tray drying (Harbourne et al., 2009). It was found that freeze drying of raspberry fruits much lower polyphenol retention compared with other drying methods such as hot air drying, infrared radiation drying, and hot air combined explosion puffing drying (Si et al., 2016). The use of low-temperature dehydration led to more serious degradation of polyphenols, which was understandable from the long drying time of 29.67±0.58 h. VD and MVD led to more higher total phenolic content, which can be explained that higher temperature drying caused more damage to the tissue, so that more phenolic compounds to be extracted or caused changes in other compounds, thereby producing more total phenols (Si et al., 2016). There was no significant difference between MVD and VD (p>0.05).

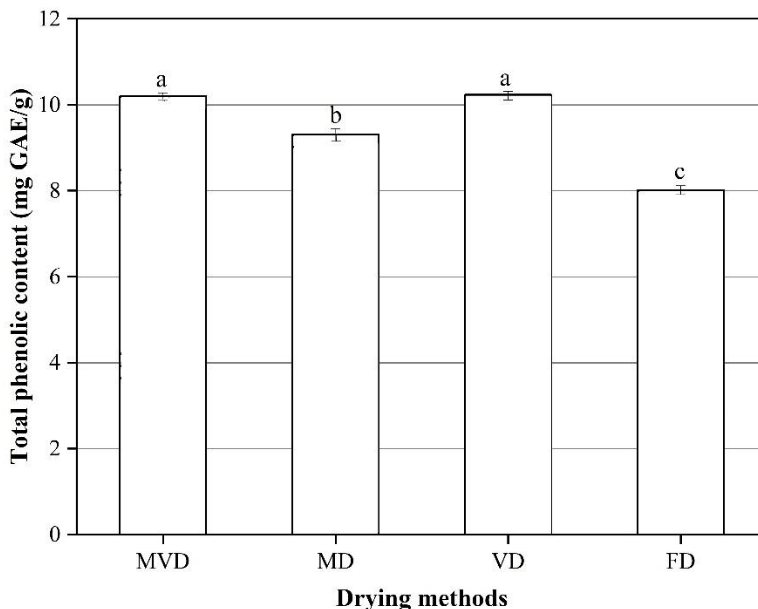

Figure 3. Total phenolic content of dried beetroots as affected by different drying methods

Effect of different drying methods on the antioxidant activities of beetroots

Beetroot contains a variety of phytochemicals with high antioxidant activity. At least two methods should be employed to evaluate the total antioxidant capacity due to different mechanisms of antioxidants, such as reducing capacity, decomposition of peroxides, free radical scavenging, and binding of transition-metal ion catalysts (Mao et al., 2006; Inchuen et al., 2010). In this study, the antioxidant activity of dried beetroots was evaluated by three methods: DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and ABTS radical scavenging activity.

The DPPH radical scavenging activity of dried beetroots is presented in Figure 4. The beetroots prepared by FD were found to show the weakest DPPH radical scavenging activity (5.16±0.02 mg TE/g). The beetroots prepared by MD and MVD showed significantly ($p<0.05$) higher DPPH radical scavenging activity than the beetroots prepared by VD and FD.

The ABTS radical scavenging activity of dried beetroots is illustrated in Figure 5. Among the dried beetroots, MD beetroots showed the highest ABTS radical scavenging activity (17.22±0.35 mg TE/g), followed by MVD, VD and FD. The result was in agreement with the results reported by Nistor et al., 2017 showed that betacyanin degradation leads to other phenolic compounds, thereby increasing antioxidant activity. The beetroots prepared by MD showed the lowest betacyanin content (Figure 1), while its ABTS radical scavenging activity was the highest. Compared to other drying methods, FD led to the lowest ABTS radical scavenging activity. It has been reported that the ABTS radical scavenging ability of freeze-dried sour cherries was significantly lower than that of vacuum microwave-dried sour cherries under several drying conditions (Wojdyło et al., 2014).

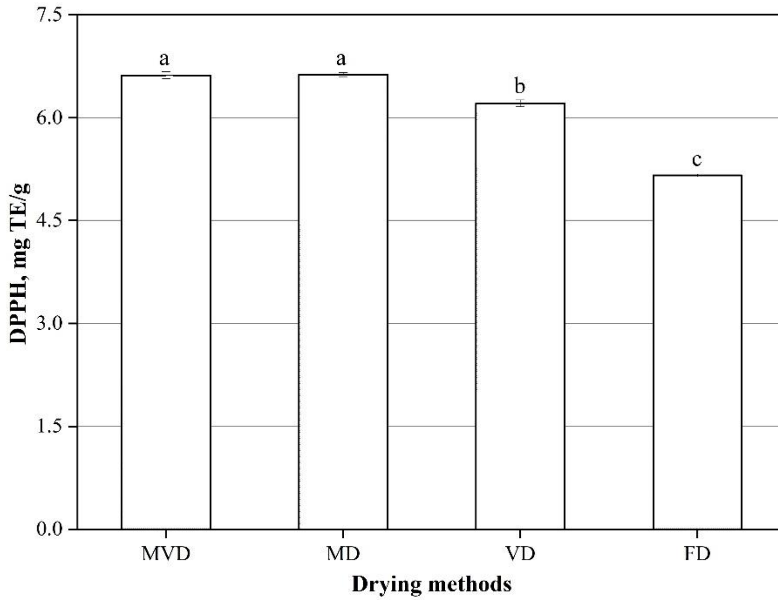

Figure 4. Effect of drying methods on the DPPH radical scavenging activity of dried beetroots

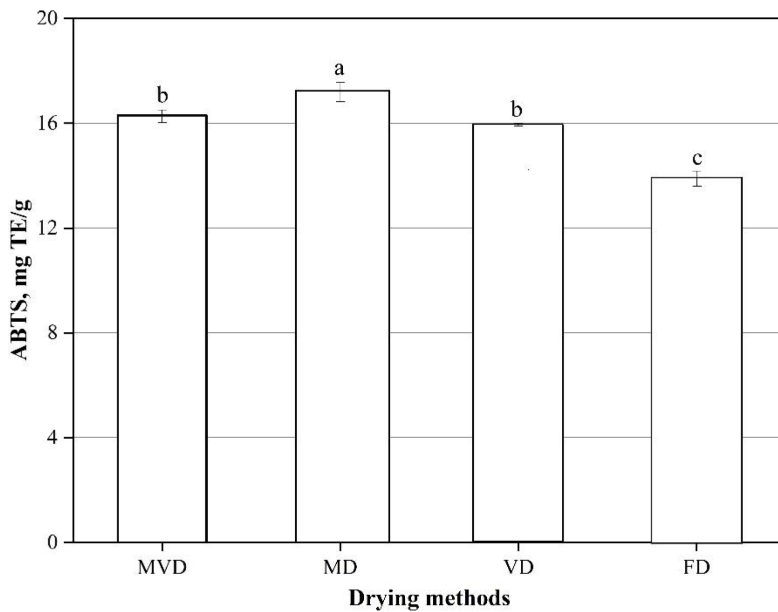

Figure 5. Effect of drying methods on the ABTS radical scavenging activity of dried beetroots

The FRAP values of dried beetroots can be seen in Figure 6. Drying methods caused the degradation of bioactive compounds that were responsible for FRAP in beetroots to different degrees. Similar to DPPH radical scavenging activity and ABTS radical scavenging activity, the beetroots obtained by FD showed the lowest FRAP value (9.89±0.13 mg TE/g).

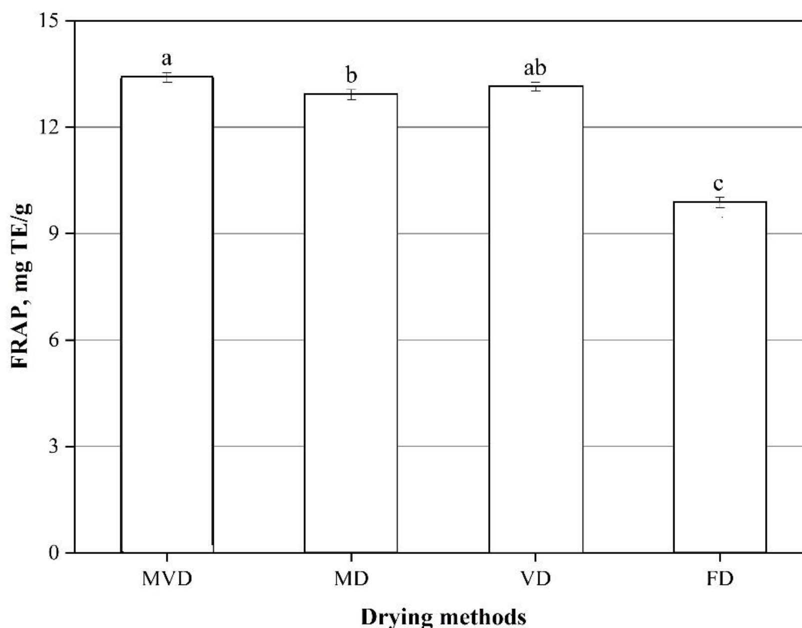

Figure 6. Effect of drying methods on the FRAP of dried beetroots

Through the antioxidant activity data, it was found that FD did not exhibit any advantages in the antioxidant activity of beetroots in comparison with the other drying methods. This result was in agreement with results published by Si et al., 2016, but contradicted that of Chan et al., 2009. Chan et al. reported that sun drying, oven drying, and microwave drying, caused more serious losses of antioxidant capacity than freeze drying. The change of antioxidant activity can be attributed to a variety of reasons. Firstly, during the thermal process, intense and/or long-term heat treatment may lead to the loss of natural antioxidants, most of which are relatively unstable (Lim et al., 2007). Moreover, degradation caused by enzymes or heating leads to the loss of antioxidant ability. In addition, the intermediate products of degradation and Maillard reaction during heating process can enhance the antioxidant capacity (Cheigh et al., 1995). The enzymatic and non-enzymatic browning reactions during drying process may also result in antioxidative characteristics (Samoticha et al., 2016).

Conclusions

The drying time, bioactive compounds and antioxidant activity of beetroots were greatly affected by drying methods. Among the four different drying methods, FD led to the longest drying time, while MVD required the shortest drying time of 0.56±0.01 h. There was no

significant difference in final moisture content of beetroots among all drying methods. In terms of bioactive compounds, the beetroots dried by FD displayed the highest betaxanthin content, while the beetroots obtained by MD showed the lowest betacyanin content. Besides, the beetroots prepared by VD showed the highest ascorbic acid content of 8.73±0.23 mg/g. Meanwhile, FD led to significantly lower total phenolic content of beetroots in comparison with other drying methods. For antioxidant activity, the beetroots prepared by MD and MVD showed significantly higher DPPH radical scavenging activity than the beetroots prepared by VD and FD. Besides, the beetroots prepared by VD exhibited the largest ABTS radical scavenging activity. Moreover, the beetroots obtained by MVD exhibited the highest FRAP value. It is noteworthy that the antioxidant activity of beetroots prepared by FD was the weakest, with the lowest values of FRAP, DPPH and ABTS. MVD led to higher antioxidant activity of beetroots in comparison with other drying methods. Comprehensive consideration of the drying time, bioactive compounds and antioxidant activity of beetroots, MVD is a suitable method for preparing dried beetroots. Dried beetroots with a large number of bioactive compounds and antioxidant activity can be utilized as food additives, functional foods and high value-added food products.

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Ethapolan synthesis by *Acinetobacter* sp. IMV B-7005 on the mixture of C2-C6-substrates and waste sunflower oil

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Abstract

Introduction. The cultivation conditions of *Acinetobacter* sp. IMV B-7005, which would provide maximum synthesis of the microbial exopolysaccharide (EPS) ethapolan on the mixture of C_2-C_6–substrates (molasses, acetate, or ethanol) and waste sunflower oil was studied.

Materials and methods. Strain IMV B-7005 was grown in liquid mineral media, containing the mixture of C_2-C_6-substrates and sunflower oil of various quality (refined, waste oil after frying potatoes, meat, vegetables, or mixed one after frying various foods). The EPS concentration was determined gravimetrically after precipitation with isopropanol, the EPS-synthesizing ability – as the ratio of the EPS concentration to the concentration of biomass and expressed in g EPS/g biomass.

Results and discussion. After replacement of refined sunflower oil in the mixture with molasses on various types of waste one (after frying potatoes, meat, vegetables, or mixed oil after frying various foods) the concentration of synthesized ethapolan was 10-14 g/l. Further neutralization of the hydrolyzed molasses with a simultaneous increase in the concentration of monosubstrates in the mixture to 3.0% was accompanied by an increase in the amount of synthesized EPS to 15.3–16.3 g/l, which remained at the same level regardless of the presence in the mixed substrate of different batches of mixed waste oil. Maximum indicators of ethapolan synthesis (15.6-15.9 g/l) on the mixture of ethanol and waste oil were achieved in the case of increasing in the culture medium content of magnesium cations (activator of key enzyme of C_2-metabolism in the producer of ethapolan) from 1.5 to 5 mM and fractional application of substrates (4 or 5 portions). Replacement of the refined substrate on mixed waste oil after frying various foods in the mixture with sodium acetate was accompanied by a slight decrease in the amount of synthesized ethapolan (from 17.3 to 16.4 g/l). However, EPS-synthesizing ability reached 7.3 g EPS/ g biomass, which was 3.7 and 2.2 times higher than the indicators obtained during the cultivation of ethapolan producer on the mixture of mixed waste oil with molasses and ethanol, respectively.

Conclusions. The obtained results are the basis for the development of universal technology for the ethapolan synthesis on mixed substrates, independent of the supplier and the quality of waste sunflower oil.

Introduction

Fierce competition in the global market of microbial polysaccharides greatly complicates the possibility of successful commercial implementation of new alternative exopolysaccharides (EPS) (Rana et al., 2020; Zayed et al., 2021). Thus, for decades, the most well-known representative of these biopolymers, which currently reaches nearly 6% of their world market, is xanthan. Several other polysaccharides have also become widespread: dextran, pullulan, levan, alginate, scleroglucan, velan, hyaluronic acid, etc. (Barcelos et al., 2020).

Although new polymers may have unique properties due to their high cost and low yield, they cannot compete with existing successful EPS.

The main ways to solve these problems are to intensify the synthesis of the target product and reduce the cost of the main components that affect its cost, in particular, the cost of carbon and energy sources.

Maximum EPS synthesis can be ensured by:

1. Increasing the efficiency of substrate conversion into the final product;
2. Optimization of cultivation conditions (Suryawanshi et al., 2019);
3. Improvement of producer strains by methods of metabolic and genetic engineering (Sun et al., 2021).

A promising approach that allows achieving a significant reduction in unproductive carbon and energy costs and intensifying the synthesis of secondary metabolites with minimal labor costs is the use of mixed substrates (Pidhorskyy et al., 2010; Pirog et al., 2013; Liu et al., 2020).

Previous studies (Pidhorskyy et al., 2010) have shown the possibility of intensifying the synthesis of exopolysaccharide ethapolan (producer of the strain *Acinetobacter* sp. IMV B-7005) on the combination of energy excess (ethanol and glucose, fumarate and glucose) and energy deficient (acetate and glucose) growth substrates. Subsequent studies have allowed to replace glucose in mixed C_2-C_6–substrates with molasses (by-product of sugar production) (Pirog et al., 2017).

Additional reduction of the cost of microbial polysaccharides is possible due to the replacement of classical expensive carbohydrate substrates on the cheap ones (Singh et al., 2019; 0et al., 2020).

One of such promising substrates is waste vegetable oil (sunflower, olive, corn, etc.), which due to its toxicity (contains carcinogens, toxic chemicals, etc. (0, 0)) cannot be reused in the food industry (food preparation, production of food additives) and agriculture (production of feed additives) (0et al., 2019).

Unfortunately, most of this waste is currently drained uncontrollably into the sewer, which leads to a number of economic (rapid wear of pipelines) and environmental (1 liter of waste oil can contaminate up to 500 thousand liters of water) problems (0et al., 2019; Loizides 0). At the same time, significant global volumes of waste oil generation (16.5 million tons per year) and the fact that its emissions to the environment are strictly regulated only in highly developed countries (USA, Japan, European countries etc.) makes the effective disposal and processing of this waste is an urgent problem (0; Mannu et al., 2020).

Currently, waste oil is usually used for biodiesel production and livestock fattening (0). Relatively new approaches of its utilization are the production of hydrogen gas, pyrolytic oil, rubber and electricity generation (Mannu et al., 2020). The main disadvantage of these approaches is the need for additional stages of pre-treatment that significantly increase the overall costs of waste oil utilization.

Another promising way to process this waste is its use in biotechnology as a substrate for the synthesis of practically valuable microbial products (surfactants, polyhydroxyalkanoates, organic acids, enzymes, vitamins, etc.) (0et al., 2020; Orjuela et al., 2020).

It should be noted that to date, information about the use of oil-containing substrates for the microbial EPS biosynthesis is quite limited, and in the available literature, the concentration of the target product does not exceed a few grams per liter (Pirog et al., 2021).

Recently, due to the significant emulsifying properties and structural features of the microbial polysaccharide ethapolan (containing residues of palmitic, oleic, palmitoleic, lauric and stearic acids) the substrates base for its production was expanded through the use of oil-containing raw materials (refined and various types of waste sunflower, corn, olive, rapeseed oil, and their mixtures) (Voronenko et al., 2020).

The purpose of this work is to establish the cultivation conditions of *Acinetobacter* sp. IMV B-7005, which would provide maximum synthesis of the microbial polysaccharide ethapolan on the mixture of C_2-C_6–substrates (molasses, acetate, or ethanol) and waste sunflower oil.

Materials and methods

Materials

For the microbial polysaccharide ethapolan production the EPS-synthesizing strain *Acinetobacter* sp. 12S, deposited in the Depository of Institute of Microbiology and Virology (National Academy of Sciences of Ukraine) under the number IMV B-7005, was used (Pidhorskyy et al., 2010).

Medium composition and cultivation conditions

The strain was grown in such liquid mineral medium (g/l):

- *Medium 1 (basic)*: KH_2PO_4 – 6.8; KOH – 0.9; NH_4NO_3 – 0.2; $MgSO_4$×7 H_2O – 0.4 (1.6 mM); $CaCl_2$×2H_2O – 0.1; $FeSO_4$×7 H_2O – 0.001;
- *Medium 2* was similar to medium 1, but NH_4NO_3 was absent;
- *Medium 3* was similar to medium 1, but NH_4NO_3 (0.2 g/l) was replaced with KNO_3 (1.5 g/l);
- *Medium 4* was similar to medium 3, but the concentration of $MgSO_4$×7 H_2O was raised to 1.25 g/l (5.0 mM).
- *Medium 5* was similar to medium 1, but NH_4NO_3 (0.2 g/l) was replaced with NH_4Cl (1.5 g/l), KH_2PO_4 concentration was decreased to 3.4 g/l and KOH was absent.

Table 1

Characteristics of *Acinetobacter* sp. IMV B-7005 growth medium

Medium number	Nitrogen source	Concentration, g/l			
		Nitrogen source	KH_2PO_4	KOH	$MgSO_4$×7 H_2O
1	NH_4NO_3	0.2	6.8	0.9	0.4 (1.6 mM)
2		0	6.8	0.9	0.4 (1.6 mM)
3	KNO_3	1.5	6.8	0.9	0.4 (1.6 mM)
4		1.5	6.8	0.9	1.25 (5.0 mM)
5	NH_4Cl	0.8	3.4	0	0.4 (1.6 mM)

An additional yeast autolysate (0.5%, v/v) was added into the medium, as well as the multivitamin complex “Complevit” (0.00085, w/w by pantothenate) (Pidhorskyy et al., 2010).

The following mixed substrates were used as a carbon and energy source: the mixture of molasses (1.5-3.0%, w/w by carbohydrates) and oil (1.5-3.0%, v/v); ethanol (4.0%, v/v) and oil (1.2%, v/v); sodium acetate (3.0%, w/w) and oil (1.5%, v/v).

In some variants refined sunflower oil was replaced with various waste oils: after frying vegetables (obtained at home after three cycles of frying for 20 minutes each), after frying potatoes and meat (from “McDonald’s” restaurant network, Kyiv), and mixed waste oil after frying various foods (meat, potatoes, onions, and cheese; from “RockerPub”, Kyiv).

In some variants, fed-batch cultivation was carried out. For that purpose the initial concentration of acetate and oil in the medium was 1.0 and 0.5% respectively, and during the cultivation these substrates were fractionally added in two portions of 1.0% (acetate) and 0.5% (oil). In one variant, acetate was replaced with an equimolar concentration of acetic acid (0.35%).

In other variant, the initial concentration of ethanol and oil in the medium was 0.8-1.0% and 0.24-0.3% respectively. During the cultivation these substrates were fractionally applied in portions (total 3-4 portions) of 0.8-1.0% (ethanol) and 0.24-0.3% (oil).

Substrates were added every 24 hours from the beginning of the cultivation process until the final concentration of mixed substrate was reached.

As inoculum was used the culture in exponential growth phase, grown in the medium with monosubstrates: 0.5 % refined and waste sunflower oil, or 0.5 % ethanol. Concentration of inoculum was 10% (Pidhorskyy et al., 2010).

Cultivation of IMV B-7005 strain was carried out in the flasks (750 ml) with 100 ml of medium in shaker (320 rpm) at 30 °C for 120 hours (Pidhorskyy et al., 2010).

Biomass and ethapolan estimation

Biomass concentration was determined by optical density of cell suspension with subsequent recalculation to dry biomass in accordance with the calibration curve (Pidhorskyy et al., 2010).

The amount of EPS was determined gravimetrically (Pidhorskyy et al., 2010). For this purpose, 1.5-2.0 volumes of isopropanol were added to a certain volume of the culture fluid (usually 10-15 ml). The polysaccharide precipitate was washed with pure isopropanol and dried at room temperature for 24 hours.

EPS-synthesizing ability was calculated as the ratio of the polysaccharide concentration to the biomass and expressed in g EPS/g biomass (Pidhorskyy et al., 2010).

Statistical data processing

All experiments were conducted in three repetitions; the number of parallel definitions in the experiments was from three to five. Statistical processing of experimental data was carried out as described earlier (Pidhorskyy et al., 2010). Differences in average indicators were considered reliable at the level of significance $p < 0.05$.

Results and discussion

Influence of the type of waste oil in the mixture with molasses on the polysaccharide synthesis

One of the promising substrates for the ethapolan synthesis is the mixture of molasses and refined sunflower oil. In previous studies (Pirog et al., 2017) the possibility of synthesizing 10 g/l EPS on the mixture of molasses (1.5%, by carbohydrates) and oil (1.5%) was demonstrated.

To further reduce the cost of carbon and energy source, the possibility of replacing refined oil on the waste one in the mixture with molasses was investigated. In these studies, the inoculum was grown on the appropriate waste oil. It is noteworthy that such oil does not require sterilization and is currently much cheaper than molasses.

Experiments have shown that when replacing refined oil in the mixed substrate with different types of waste one (after frying potatoes, meat, vegetables, or mixed waste oil after frying various foods) the concentration of synthesized ethapolan (10–14 g/l) was the same as when using a refined substrate (10–12.5 g/l) (Table 2).

It should be noted that from a practical and economic point of view, for the inoculum preparation and EPS biosynthesis, it is most appropriate to use mixed waste oil, because before sending for disposal, different batches of oil after frying different foods are usually mixed.

Table 2

Ethapolan biosynthesis on mixture of different type of waste oil (1.5%) and molasses (1.5%)

Type of oil in the mixture with molasses	EPS, g/l	EPS-synthesizing ability, g EPS/g biomass
Refined (control)	10.09±0.50	3.60±0.18
Waste oil after frying potato (fries)	11.06±0.55	2.61±0.13
Waste oil after frying potato (wedges)	11.66±0.58	2.69±0.13
Waste oil after frying meat	12.41±0.62	3.42±0.17
Waste oil after frying vegetables	9.94±0.50	2.95±0.15
Mixed waste oil after frying various foods	13.92±0.70	3.49±0.17

Note. The inoculum was grown on medium 1, EPS biosynthesis on medium 2. The inoculum was grown on the refined or appropriate type of waste oil.

Since *Acinetobacter* sp. IMV B-7005 enzyme systems involved in sucrose catabolism have low activity in these studies molasses was hydrolyze before usage. The pH of the substrate was reduced to 4.0–4.5, which led to a decrease in the initial pH of the culture fluid to 5.8–6.0, which are suboptimal for the growth of the producer and the ethapolan synthesis (Pidhorskyy et al., 2010).

It was previously shown that the use of neutralized after hydrolysis molasses in the mixture with C_2-substrates (ethanol or acetate) allowed not only to avoid excessive decrease of the initial pH of the culture fluid, but was also accompanied by an additional increase in the synthesis of ethapolan (Pidhorskyy et al., 2010).

Therefore the effect of the method of molasses preparation on the synthesis of EPS during growth of strain IMV B-7005 on its mixture with waste oil was further investigated.

Experiments showed that regardless of the content of neutralized molasses and oil in the mixture (1.5–3.0%) the amount of synthesized polysaccharide was 1.15–1.25 times higher than the concentration obtained with the use of conventional hydrolyzed substrate (Table 3).

Table 3

Indicators of ethapolan synthesis on the mixture of molasses and oil depending on the method of molasses preparation

Method of molasses preparation	Concentration of monosubstrates in the mixture, %		pH_{end}	EPS, g/l	EPS-synthesizing ability, g EPS/g biomass
	Molasses	Oil			
Hydrolyzed without neutralization (control)	1.5	Refined, 1.5	6.9	10.09±0.50	3.60±0.18
Neutralized after hydrolysis	1.5	Refined, 1.5	7.0	12.15±0.61	2.25±0.11
	1.5	Mixed waste (1)*, 1.5	7.0	11.28±0.56	1.43±0.07
	1.5	Mixed waste (2)*, 1.5	7.2	11.73±0.59	2.16±0.11
	1.5	Mixed waste (3)*, 1.5	7.2	10.28±0.51	1.17±0.06
Hydrolyzed without neutralization (control)	2.0	Refined, 2.0	6.9	11.54±0.58	3.02±0.15
Neutralized after hydrolysis	2.0	Refined, 2.0	7.0	14.45±0.72	2.59±0.13
	2.0	Mixed waste (1)*, 2.0	7.1	12.23±0.61	1.29±0.06
	2.0	Mixed waste (2)*, 2.0	7.2	12.93±0.65	1.78±0.09
	2.0	Mixed waste (3)*, 2.0	7.3	13.51±0.68	1.39±0.07
Hydrolyzed without neutralization (control)	3.0	Refined, 3.0	6.8	13.09±0.65	3.11±0.16
Neutralized after hydrolysis	3.0	Refined, 3.0	7.3	16.25±0.81	1.95±0.10
	3.0	Mixed waste (1)*, 3.0	7.4	16.02±0.80	1.96±0.10
	3.0	Mixed waste (2)*, 3.0	7.6	15.28±0.76	1.47±0.07
	3.0	Mixed waste (3)*, 3.0	7.4	15.28±0.76	1.50±0.08

Note. The inoculum was grown on medium 1, EPS biosynthesis on medium 2. The inoculum was grown on the refined or mixed waste oil after frying various foods was used. * – the batch number of mixed waste oil after frying various foods was used is given in brackets.

It should be noted that the indicators of ethapolan synthesis on different batches of mixed waste oil after frying various foods did not change for the same concentration of monosubstrates in the mixture. A slight decrease in the amount of synthesized ethapolan and EPS-synthesizing ability compared to the use of refined substrate may be due to the presence in the waste oil of various toxic compounds (aldehydes, free radicals, etc.), which are formed during frying (0).

In general, the highest rates of ethapolan synthesis (concentration of EPS was 15.3–16.3 g/l, EPS-synthesizing capacity was 1.5–2.0 g EPS/g biomass) were observed with the use of 3.0% (by carbohydrates) neutralized molasses in the mixture with 3.0% of refined or mixed waste oil after frying various foods.

Note that various methods of molasses pre-treatment are widely used to increase the EPS synthesis on this substrate (0et al., 2019; Ai et al., 2015).

It is known that molasses contains water, sucrose (45-55%), organic acids, amino acids, vitamins, and minerals, metal cations (Palmonari et al., 2020). Most of these compounds promote the growth of microorganisms and the synthesis of the final product, but, on the other hand, the presence of heavy metals in the culture medium can cause certain problems during biosynthesis. In particular, they are able to inhibit the growth of microorganisms and lead to the inactivation of enzymes involved in the synthesis of the target product (Ai et al., 2015). At the same time, such inhibitors can be partially removed by pre-treatment of molasses with acid, activated carbon, calcium phosphate, etc. (Li et al., 2020; Gojgic-Cvijovic et al., 2019).

Replacement in the mixture with C_2-substrates of refined oil on mixed waste oil after frying various foods

It should be noted that during growth of the IMV B-7005 strain on the mixture of molasses and oil, there was a decrease in EPS-synthesizing capacity by almost 2 times compared to the use of oil as monosubstrate. We have suggested that this may be due to the insufficient C/N ratio due to the presence of additional nitrogen in the molasses.

Therefore, in the next stage, the possibility of ethapolan synthesis on the mixture of waste oil and substrate that do not contain nitrogen, in particular ethanol or acetate, was investigated. Due to the peculiarities of the pH change of the culture fluid during the cultivation of the IMV B-7005 strain on the mixture of refined oil and C_2-substrates (Pidhorskyy et al., 2010) fed-batch cultivation was carried out.

In subsequent experiments during refined oil replacement in the mixture with ethanol on mixed waste oil after frying various foods additional increase of the concentration of Mg^{2+} cations in the culture medium was applied. These cations are one of the activators of acetyl-CoA synthetase – key enzyme C_2-metabolism in *Acinetobacter* sp. IMV B-7005. Low activity of acetyl-CoA synthetase in the IMV B-7005 strain is one of the reasons of acetate metabolism limitation.

It was found that when the content of Mg^{2+} increased to 5 mM indicators of ethapolan synthesis (concentration of EPS was 15.6–15.9 g/l, EPS-synthesizing capacity was 3.1–3.3 g EPS/g biomass) on the mixture of ethanol and mixed waste oil after frying various foods did not depend on the method of fractional application of substrates (4 or 5 portions) and were higher than results obtained on the mixture of ethanol and refined oil (10–13.5 g/l) (Table 4).

At the same time, the additional introduction of Mg^{2+} in the medium with ethanol and refined oil led to a slight decrease in the synthesis of EPS. We suggest that this may be due to the different effects of these cations on the enzymatic systems responsible for the catabolism of fatty acids and other related components that are part of sunflower oil or formed during frying. Li et al. (2014) found that in lactic acid bacteria *Lactococcus lactis* ssp. *lactis* concentration of Mg^{2+}, at which the maximum enzymatic activity is observed, differs for various enzymes of the β-oxidation system.

Table 4

Influence of magnesium cations concentration on the ethapolan synthesis on the mixture of ethanol (4.0%) and sunflower oil (1.2%) of different quality

Concentration of Mg^{2+} in the medium, mM	Type of oil in the mixture with ethanol	Mode of fed-batch cultivation	pH_{end}	EPS, g/l	EPS-synthesizing ability, g EPS/ g biomass
1.6 (medium 3)	Refined (control)	Four portions*	5.9	11.88±0.59	3.08±0.15
		Five portions**	6.1	13.51±0.68	3.65±0.18
	Mixed waste oil after frying various foods	Four portions*	6.2	12.02±0.60	3.24±0.16
		Five portions**	6.1	10.10±0.51	2.73±0.14
5.0 (medium 4)	Refined (control)	Four portions*	6.1	10.37±0.52	2.58±0.13
		Five portions**	6.2	9.86±0.49	2.19±0.11
	Mixed waste oil after frying various foods	Four portions*	6.0	15.63±0.78	3.33±0.17
		Five portions**	5.9	15.95±0.80	3.11±0.16

Notes: The inoculum was grown on ethanol. Fractional application of substrates was carried out 4-5 times every 24 hours: * – four portions of 1.0% ethanol and 0.3% oil; ** – five portions of 0.8% ethanol and 0.24% oil.

Further experiments have shown (Table 5) that after the replacement of refined oil in the mixture with acetate on mixed waste oil after frying various foods the amount of final product reached 16.36 g/l, which was only slightly lower than that obtained using a refined substrate (17.27 g/l). Meanwhile, EPS-synthesizing capacity increased up to 7.34 g EPS/g biomass, that was 3.7 and 2.2 times higher than the indicators obtained on the mixture of mixed waste oil after frying various products and molasses or ethanol, respectively.

Table 5

Biosynthesis of ethapolan on the mixture of sodium acetate (3.0%) and sunflower oil (1.5%) of different quality

Type of oil in the mixture with sodium acetate	pH_{end}	EPS, g/l	EPS-synthesizing ability, g EPS/g biomass
Refined (control)	7.9	17.27±0.86	6.47±0.32
Mixed waste oil after frying various foods	7.7	16.36±0.82	7.34±0.37

Notes: Inoculate cultivation and EPS biosynthesis were carried out on medium 5. The inoculum was grown on the refined or mixed waste oil after frying various foods. Fractional application of substrates was carried out three times every 24 hours: two portions of 1.0% acetate and 0.5% of oil, and one portion of 0.35% acetic acid and 0.5% of oil.

Conclusion

Thus, as the result of that research:

1. The possibility of replacing refined sunflower oil with different types of waste one, in particular mixed waste oil after frying various foods, in the mixture with C_2-C_6–substrates (molasses, ethanol or sodium acetate) for the ethapolan synthesis was demonstrated;
2. Optimal cultivation conditions (concentration of substrates in the mixture of molasses and oil, method of fractional application of substrates during growth on the mixture of oil with ethanol or sodium acetate) providing the maximum synthesis of the final product on the corresponding mixed substrates were established;
3. It was found that regardless of the type of used C_2-C_6–substrates in the mixture with waste oil, the concentration of ethapolan reached 15.3–16.3 g/l;
4. Data obtained are the basis for the development of a universal technology for the ethapolan synthesis on mixed substrates, independent of the supplier and the quality of waste sunflower oil.

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Анотації

Харчові технології

Вплив мікрохвильового і гідротермічного оброблення на колірні характеристики, розмір частинок і розподіл води в картопляному крохмалі

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Вступ. Метою дослідження є визначення впливу мікрохвильового і гігротермічного оброблення на колірні характеристики, розмір частинок і розподіл води в картопляному крохмалі.

Матеріали і методи. Нативний картопляний крохмаль модифікували у полі НВЧ-випромінювання і гідротермічними обробленням. Проводилися таке оброблення картопляного крохмалю: одноразове мікрохвильове оброблення (НВЧ); гідротермічне оброблення за допомогою попереднього НВЧ оброблення (НВЧ-ГТО) і термо- та вологообробка за допомогою мікрохвильової післяобробки (ГТО -НВЧ).

Результати і обговорення. Загалом, ГТО спричинило невелике збільшення яскравості (значення L*), тоді як одиночне НВЧ – невелике зниження яскравості.У результаті колір усіх оброблених ГТО зразків став яскравішим, а колір одиночного зразка, обробленого НВЧ (НВЧО), став темнішим. Колір зразків НВЧ-ГТО став більш червоним, а зразків НВЧО, ГТО та ГТО-НВЧ – більш зеленим. Хоча були значні відмінності в різниці кольору (ΔE), але можна констатувати, що всі методи оброблення несуттєво змінили колір картопляного крохмалю, при цьому ΔE завжди нижче 5. Середній діаметр (D50), діаметр частинок об'єму (D(4,3)) та діаметр частинок поверхні (D(3,2)) всього обробленого крохмалю були вищими порівняно з нативним крохмалем (НК), тоді як значення питомої поверхні значно зменшилося за допомогою НВЧ і ГТО, що вказує на те, що ці методи оброблення можуть викликати розширення, часткову клейстеризацію й агломерацію крохмальних гранул, що призводить до великого розміру частинок крохмальних гранул. Оброблення картопляного крохмалю НВЧ і ГТО спричинила зміщення часу релаксації T21 у бік більш швидких часів релаксації порівняно з природним крохмалем.

Хоча вода в усіх зразках крохмалю була основною (90%), три піки спостерігалися для часу релаксації T2 крохмалю, обробленого НВЧ (НВЧО, НВЧ-ГТО і ГТО-НВЧ), і два піки – для часу релаксації часу T2 зразків НК і ГТО. Отже, крохмаль, оброблений НВЧ, мав три різні стани води, тоді як НК і одиночний ГТО крохмаль мали лише два різних стани води. Крім того, оброблення НВЧ і ГТО можуть змінити розподіл води та покращити взаємодію між крохмалем і водою.

Висновки. Хоча оброблення НВЧ і ГТО помітно не змінили колір картопляного крохмалю, ці види оброблення можуть спричинити розширення, часткову клейстеризацію й агломерацію крохмальних гранул, що призводить до великого розміру частинок крохмальних гранул. Оброблення НВЧ і ГТО може змінити розподіл води та покращити взаємодію між крохмалем і водою.

Ключові слова: *картопляний крохмаль, НВЧ, гідротермічний, колір, розмір.*

Часткове очищення і характеристика поліфенолоксидази з оболонки кінських бобів (*Vicia faba*)

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Вступ. Метою цього дослідження є виділення ферменту поліфенолоксидази (PPO) з оболонки кінських бобів, визначення його оптимального pH і температури, параметрів Km та Vmax, дослідження біохімічних властивостей, таких як температурна і кислотна стабільність, селективність субстрату, ферментна стабільність під час зберігання, а також визначення впливу деяких металів та інгібіторів на їхню активність.

Матеріали і методи. Осадження білка проводили за допомогою солі сульфату амонію. Для видалення іонів солі після осадження проводили діаліз. Вміст білка у зразках на кожному етапі визначали за методом Бредфорда, а ферментативну активність – спектрофотометрично.

Результати і обговорення. Властивості ферменту PPO, очищеного в 3,1 раза, визначали з використанням катехолу як субстрату. Значення Km та Vmax ферменту становили 5,53 мМ та 4424,58 $U.ml^{-1}$ min^{-1}, відповідно. Найвищу активність фермент виявив при pH 5,0 і 10 ºС. Він зберіг близько 60% своєї активності після 30-хвилинного періоду інкубації при оптимальному pH. Субстратну специфічність PPO вивчали з використанням восьми субстратів, включаючи пірогалол та 4-метилкатехол. Під час дослідження в діапазоні температур 10–60 ºС профіль термічної стабільності виявив більше 50% своєї активності в діапазоні 10–40 ºС після однієї години інкубації. У той час як L-аскорбінова кислота, сульфіт натрію і L-цистеїн виявляли сильний інгібувальний ефект, досліджувані метали демонстрували різний вплив на активність ферменту.

Висновки. Активність ферменту поліфенолоксидази викликає небажані зміни та втрату харчової цінності деяких фруктів і овочів. Отримані результати дають інформацію про PPO кінських бобів та ефективні методи контролю потемніння під час зберігання.

Ключові слова: *кінський біб, очищення, поліфенолоксидаза, стабільність.*

Застосування високого тиску в технологіях гелевих м'ясних продуктах з низьким вмістом солі. Огляд

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Вступ. Метою статті є аналіз і узагальнення дослідження впливу високого тиску на гелеві м'ясні продукти з низьким вмістом солі (1%); зміна структури білків м'яса і властивостей гелю.

Матеріали і методи. Аналіз літератури.

Результати і обговорення. Високий тиск як різновид технології фізичної стерилізації, може ефективно змінювати структуру білка, технологічні й функціональні характеристики м'ясних продуктів, зберігаючи при цьому поживні властивості і смак. Розглянуто зміну водоутримувальної здатності, кольору, текстури і сенсорних властивостей м'ясних гелевих продуктів з низьким вмістом солі під високим тиском. Проаналізовано, як впливають параметри технологічного процесу (тиск, час і температура) на зміну структури, конформації і властивостей гелю м'ясних гелевидних продуктів з низьким вмістом солі . М'ясний білок чутливий до високого тиску. Структури α-спіралі і β-листа змінилися на структури випадкових спіралей і β-витків у міру збільшення тиску. Розчинність білка і твердість гелю досягають своїх максимальних показників, а мікроструктура гелю оптимально щільна і однорідна при 200 МПа. В цілому, обробка високим тиском може використовуватися для виробництва гелевих м'ясних продуктів з низьким вмістом солі і забезпечувати їх високу якість. Обробка яловичої ковбаси з низьким вмістом солі при високому тиску 200 МПа призводить до підвищеної розчинності міофібрилярних білків, таких як міозин і актин, в солях. Комбінація високого тиску і теплової обробки при низьких температурах денатурації білків солоного м'яса призводить до кращого утримання води і текстури, ніж у зразків, приготованих тільки шляхом нагрівання. Отже, обробка високим тиском перед нагріванням покращує функціональність м'ясного фаршу.

Висновки. Обробка високим тиском може бути ефективно використана при виробництві гелеподібних м'ясних продуктів з низьким вмістом солі і високими споживчими якостями.

Ключові слова: *м'ясо, гель, високий тиск, сіль, водоутримувальна здатність, текстура.*

Вплив часу пропарювання на колір і фізичні властивості чотирьох сортів рису, вирощуваних у Західній Африці

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Вступ. Гідротермічне оброблення під час пропарювання викликає желатинізацію та розпад білка в ендоспермі, змінюючи зовнішній вигляд і міцність продукту за рахунок сплавлених клейстеризованих крохмальних гранул, які руйнують білкові тіла.

Матеріали і методи. Оцінювали періоди гідротермічного оброблення на міцність зерна і зовнішній вигляд сортів рису FARO64, FARO65, FARO66 і FARO67. Кожен сорт поділяли на п'ять частин; чотири частини пропарювали і піддавали гідротермічному обробленню протягом 5, 10, 15 і 20 хв (за постійної температури та атмосферному тиску), а п'ята частина була контрольною.

Результати і обговорення. Внесення пари мало достовірний вплив ($p<0,05$) на міцність зерна. Оптимальна міцність була зафіксована при 20-хвилинному періоді гідротермічного оброблення у сортів FARO64, FARO65 і FARO66, тоді як FARO67 мав найвищу міцність на 15-й хвилині із значеннями від 61,53N до 225,83N. FARO65 мав знижене значення кольору L*, хоча всі сорти зберегли жовтий колір під час гідротермічного оброблення. Лише FARO64 підтримував значення кольору a* під час

гідротермічного оброблення. Що стосується довжини, сорт FARO67 змінився на дуже довгий, а FARO64, FARO65 і FARO66 змінився із середньої на довгий з діапазоном значень від 6,33 мм до 7,57 мм.

Значення ширини (1,98-2,62 мм) змінювалися однаково для всіх сортів. Усі сорти, окрім FARO64, зменшені в товщині під час гідротермічного оброблення. Форма сорту FARO64 змінилася із середньої на тонку; сорти FARO65 і FARO66 зберегли середню форму, а FARO67 – змінилася від тонкої до середньої. Маса тисячі зерен була від 15 до 24; сорти FARO64, FARO66 і FARO67 мали знижене середнє значення, тоді як FARO65 – зросла.

Висновок. Пропарювання зменшує швидкість руйнування сирого рису, покращує міцність, колір і зовнішній вигляд. Температура желатинізації впливає на якість рису.

Ключові слова: *рис, гідротермічне оброблення, пропарювання, желатинування.*

Вплив загущувачів на основі пектину і модифікованого крохмалю на структурні характеристики низькокалорійного яблучного джему

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Вступ. Метою дослідження є визначення впливу загущувачів на основі пектину і модифікованого крохмалю на структурні характеристики джему низькокалорійного.

Матеріали і методи. Досліджувалася технологія джему на основі яблучного пюре з повним вилученням цукру рафінованого та з додаванням підсолоджувача. Методи дослідження: кальцій-пектатний метод визначення пектинових речовин, рефрактометричний метод визначення сухих речовин, метод визначення маси навантаження для руйнування структури драглів для визначення міцності; профільний метод за 10-бальною шкалою відповідності інтенсивності відчуття ароматичних і смакових властивостей.

Результати. Вилучення цукру рафінованого з рецептури призводить до погіршення структурних характеристик джему. Задля визначення кількісних співвідношень інгредієнтів було вивчено залежність фізико-хімічних показників якості джему від вмісту загущувачів у ньому.

Зменшення кількості цукру в межах 100–333 г/1000 г призводить до зменшення міцності від 320 г до 160 г. Разом з цим втрачається значна кількість сухих речовин: від 65,2 до 45,2 %. Їх кількість частково зростає при збільшенні вмісту яблучного пюре від 550 г / 1000 г джему (зразок контрольний) до 650 г.

Це пояснює незначне збільшення титрованих кислот від 1,0 до 1,5 мг / 100 г. Показник титрованої кислотності був визначальним при виборі пектину як харчової добавки для покращення структурних характеристик готового продукту. Достатньою кількістю пектину можна вважати 10 г/1000 г джему. Міцність структури при цій кількості становить 300 г.

Встановлено доцільність використання модифікованого крохмалю кукурудзяного Pregel 200 G у кількості 10 г на 1000 г готової продукції, що сприяє створенню термозворотніх гелів і стабілізує текстуру джему, зберігаючи його міцність.

Додавання екстракту стевії в кількості 15 г/1000 г є оптимальним. Подальше

підвищення кількості екстракту стевії не є обґрунтованим, бо смак залишається інтенсивно солодкий і різниця особливо не відчувається. Профілограма смаку досліджуваних зразків демонструє збереження солодкого смаку та незначне збільшення повноти гіркого смаку.

Висновки. Додавання пектину і крохмалю як загущувачів джему, який містить сухий екстракт стевії та не містить цукру рафінованого, дає змогу отримати джем низькокалорійний і зберегти сенсорні властивості продукту.

Ключові слова: *джем, сухий екстракт стевії, пектин, модифікований крохмаль.*

Вплив плющення зерна пшениці на режими подрібнення у вальцьовому верстаті

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Вступ. Процес плющення застосовується для інтенсифікації подрібнення зерна при сортових помелах пшениці, при цьому оптимальна величина зазору між вальцями плющильної системи при помелах пшениці в сортове борошно залишається невизначеною.

Матеріали і методи. Плющення здійснювали в лабораторному плющильному верстаті з діаметром вальців 144,0 mm; шириною вальців – 68,4 mm та швидкістю обертання 14,6 s^{-1}. Подрібнення зерна пшениці здійснювали у вальцьовому верстаті. Довжина вальців – 70 мм; кількість рифлів на 1 см кола вальців – 6, ухил рифлів – 12%, співвідношення колових швидкостей – 1:2, швидкість обертання швидкохідного вальця – 3,93 м/с, діаметр вальців – 150 мм, взаєморозташування рифлів – спинка по спинці, кути загострення рифлів – 35°/70°. Режим подрібнення зерна пшениці у плющильному верстаті та у вальцьовому верстаті визначали шляхом просіювання отриманих продуктів через сито з отворами 1,0 мм. Гранулометричний аналіз визначали шляхом просіювання на стандартному наборі сит із модулем $\Delta \approx 1{,}21$.

Результати і обговорення. При подрібненні продуктів плющення у вальцьовому верстаті загальний вихід проміжних продуктів має нелінійний характер і може бути апроксимований рівнянням другого степеня. Оптимальна величина відстані між вальцями плющильного верстату становить 1,4 mm за умови, що дрібні продукти після плющення були попередньо виділені із суміші плющеного продукту.

Сумарний добуток проміжних продуктів після плющення та подрібнення у вальцьовому верстаті має складний нелінійний характер, який не дає змоги встановити оптимальне значення величини зазору між вальцями плющильної системи. Сумарний добуток проміжних продуктів, які отримано сумарно при плющенні та подрібненні, більший на 2,9 %, ніж сумарний добуток продуктів, отриманих лише у вальцьовому верстаті з попередньо видаленими дрібними продуктами плющення.

Диференціальні криві є полімодальними і мають п’ять максимумів. Інтегральні криві мають S-подібний вид. При подрібненні цілого зерна сумарний вихід проміжних продуктів більший, ніж при подрібненні плющеного зерна у вальцьовому верстаті за однакових умов. При подрібненні плющеного зерна утворюється більша кількість крупних фракцій продуктів за рахунок дрібних.

Висновки. У діапазоні встановленого оптимального зазору між вальцями плющильного верстату значних відмінностей між двома способами подрібнення пшениці не встановлено.

Ключові слова: *пшениця, плющення, подрібнення, зазор, вальці.*

Особливості складу ліпідів у соняшниковому воску

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Вступ. Метою дослідження є визначення особливостей складу ліпідів у соняшниковому воску, виділеному з нерафінованої та рафінованої олії.

Матеріали і методи. Воски відділяли від триацилгліцеринів у колонці із силікагелем. Склад восків визначали газорідинною хроматографією з полум'яно-іонізаційним детектором. Колонку термостатували в заданому режимі в діапазоні з 170 °С до 380 °С.

Результати і обговорення. У статті подано експериментальні дані визначення складу восків у нерафінованій і рафінованій соняшниковій олії. Описані в методиці дослідження умови газорідинної хроматографії розширили діапазон виявлення довголанцюгових восків. Отримано нові дані щодо присутності восків з С50 і більше вуглецевими атомами у соняшниковій олії.

У нерафінованій соняшниковій олії ідентифіковано воскові сполуки з довжиною вуглецевоводневого ланцюга від С44 до С56 вуглецевих атомів. У соняшниковій нерафінованій олії воскових сполук із С46, С48, С50 і С52 вуглецевих атомів міститься до 61 % від загальної кількості восків. Визначені воски з парною кількістю вуглецевих атомів становили 82 % від загальної кількості восків. Найбільший вміст воску з С48 вуглецевими атомами в ланцюзі виявлено у нерафінованій соняшниковій олії.

У рафінованій соняшниковій олії вміст воскових сполук зменшився на порядок. Під час рафінування видалено воски з коротшими вуглецевоводневими ланцюгами С44 і С46. Після рафінування в олії все ж залишаються слідові кількості восків, зокрема воскові сполуки з кількістю вуглецевих атомів С48, С49, С50, С51, С52, С53, С54. Причому ланцюги з парною кількістю вуглецевих атомів мають переважний вміст над восками з непарною кількістю вуглецевих атомів у ланцюзі. У рафінованій соняшниковій олії переважають воски з С50, С52 і С54 вуглецевими атомами в ланцюзі.

Проаналізовано кислотне і йодне числа, число омилення і температура плавлення воску, які кореспондуються з визначеним складом соняшникового воску.

Висновки. Залежність вмісту восків від довжини ланцюга з парними і непарними кількостями вуглецевих атомів у нерафінованій і рафінованій соняшниковій олії є параболічною.

Ключові слова: *ліпіди, віск, соняшник, олія, ефір.*

Біоактивні сполуки та антиоксидантна активність буряків, приготованих різними методами сушіння

4

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Вступ. Метою статті є дослідження впливу різних методів сушіння, включаючи сушіння заморожуванням, вакуумне сушіння, мікрохвильове сушіння та мікрохвильове сушіння у вакуумі, на біоактивні сполуки й антиоксидантну активність буряків.

Матеріали і методи. Досліджувались біологічно активні сполуки та антиоксидантна активність сушених буряків, отриманих шляхом сублімаційного сушіння, вакуумного сушіння, мікрохвильового сушіння та мікрохвильового вакуумного сушіння. Для визначення вологості буряків використовували аналізатор вологи. Буряковий порошок тричі екстрагували 50% етанолом (об./об.). Колориметричні методи були використані для визначення вмісту беталаїну, аскорбінової кислоти, загального фенолу та 1,1-дифеніл-1-пікрилгідразилової радикальної активності, що знижує антиоксидантну здатність заліза та 2,2′-азино-біс (3-етилбензтіазолін-6-сульфонова кислота) радикальну активність поглинання сухих буряків.

Результати і обговорення. На підставі отриманих результатів встановлено, що методи сушіння значно вплинули на час висихання, біоактивні сполуки та антиоксидантну активність сушених буряків. Найкоротший час сушіння 0,56±0,01 год відбувався у мікрохвильовій сушарці з використанням вакууму, тоді як у сублімаційній сушарці час сушіння найтриваліший – 29,67±0,58 год. Не було суттєвої різниці в кінцевій вологості сушеного буряка, приготованого різними методами сушіння. Було встановлено, що сухий порошок з буряку, який був висушений у вакуумній сушарці, показав найвищий вміст аскорбінової кислоти 8,73±0,23 мг/г, а буряк, висушений у сублімаційній сушарці, показав найвищий вміст бетаксантину 2,57±0,01 мг/г, тоді як висушений буряк, отриманий за допомогою мікрохвильового випромінювання, показав найнижчий вміст бетаціаніну 2,98±0,02 мг/г. Сушіння методом сублімації призвело до значно нижчого загального вмісту фенольних речовин у буряках порівняно з іншими методами сушіння. З точки зору антиоксидантної активності висушений буряк за допомогою мікрохвильового випромінювання та мікрохвильового випромінювання з використанням вакууму демонстрував значно вищу активність очищення від радикалів 1,1-дифеніл-1-пікрилгідразил, ніж буряк, висушений у вакуумній і сублімаційній сушарках. Дані дослідження підтвердили, що при використанні вакуумного методу сушіння найбільшу активність поглинання вільних радикалів має 3-етилбензтіазолін-6-сульфонова кислота, яка становила 17,22±0,35 мг еквівалента тролоксу. Крім того, значення 2,2′-азино-біс буряка, висушеного у вакуумній мікрохвильовій сушарці, було найвищим – 13,36±0,17 мг/г. Встановлено, що при сублімаційному сушінні буряку найнижчі значення показали 2,2′-азино-біс, 1,1-дифеніл-1-пікрилгідразил та 3-етилбензтіазолін-6-сульфонова кислоти. Порівняно з іншими методами цей метод показав найнижчу антиоксидантну активність.

Висновки. Враховуючи час висихання, біоактивні сполуки та антиоксидантну активність, для приготування зневодненого буряка рекомендується мікрохвильове вакуумне сушіння, що дає змогу скоротити час сушіння, краще зберегти біоактивні сполуки тй антиоксидантну активність буряка.

Ключові слова: *буряк, сушіння, вакуум, мікрохвильовий, антиоксидант.*

Біотехнологія. Мікробіологія.

Синтез етаполану *Acinetobacter* sp. ІМВ В-7005 на суміші C_2-C_6-субстратів і відпрацьованої соняшникової олії

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Вступ. Стаття присвячена дослідженню умов культивування *Acinetobacter* sp. ІМВ В-7005, які б забезпечували максимальні показники синтезу мікробного екзополісахариду (ЕПС) етаполану на суміші C_2-C_6-субстратів (меляса, ацетат, етанол) та відпрацьованої соняшникової олії.

Матеріали і методи. Штам ІМВ В-7005 вирощували в рідких мінеральних середовищах, які містили суміш C_2-C_6-субстратів і соняшникову олію різної якості (рафіновану, відпрацьовану після смаження картоплі, м’яса, овочів або змішану після смаження різних продуктів). Концентрацію ЕПС визначали ваговим методом після осадження ізопропанолом, ЕПС-синтезувальну здатність − як відношення концентрації ЕПС до біомаси та виражали у г ЕПС/г біомаси

Результати і обговорення. Після заміни рафінованої соняшникової олії у суміші з мелясою на різні типи відпрацьованої (після смаження картоплі, м’яса, овочів або змішану після смаження різних продуктів) концентрація синтезованого етаполану становила 10-14 г/л. Подальша нейтралізація гідролізованої меляси з одночасним збільшенням концентрації моносубстратів у суміші до 3,0% супроводжувалася підвищенням кількості синтезованого ЕПС до 15,3–16,3 г/л, яка залишалася на такому самому рівні незалежно від наявності у змішаному субстраті різних партій змішаної відпрацьованої олії. Максимальні показники синтезу етаполану (15,6–15,9 г/л) на суміші етанолу та відпрацьованої олії досягалися в разі підвищення в середовищі культивування вмісту катіонів магнію (активатор ключових ферментів C_2-метаболізму у продуцента етаполану) з 1,5 до 5 мМ та дробного внесення субстратів (4 або 5 порцій). Заміна рафінованої олії на змішану відпрацьовану після смаження різних продуктів у суміші з ацетатом натрію супроводжувалось незначним зниженням кількості синтезованого етаполану (з 17,3 до 16,4 г/л). Проте при цьому ЕПС-синтезувальна здатність досягала 7,3 г ЕПС/ г біомаси, що у 3,7 та 2,2 раза вище за показники, встановлені під час культивування продуцента етаполану на суміші змішаної відпрацьованої олії з мелясою та етанолом відповідно.

Висновки. Отримані результаті є основою для розробки універсальної технології синтезу етаполану на змішаних субстратах, незалежної від постачальника та якості відпрацьованої соняшникової олії.

Ключові слова: *Acinetobacter, змішані субстрати, відпрацьована олія, біосинтез, екзополісахарид, етаполан.*

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Results and discussion describe the main findings (23-26 lines).

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Deegan C. (2000), *Financial Accounting Theory*, McGraw-Hill Book Company, Sydney.

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Arych M. (2018), Insurance's impact on food safety and food security, *Resource and Energy Saving Technologies of Production and Packing of Food Products as the Main Fundamentals of Their Competitiveness: Proceedings of the 7th International Specialized Scientific and Practical Conference, September 13, 2018,* NUFT, Kyiv, pp. 52-57.

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