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DEVELOPMENT AND QUALITY ASSESSMENT OF HEAT-STABLE FRUIT FILLINGS CONTAINING DIETARY FIBERS

JANNA CROPOTOVA

Practical Scientific Institute of Horticulture and Food Technology, 59 Vierul St., MD 2070, Chisinau, Moldova
jcropotova@gmail.com

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The study is devoted to the development and quality assessment of fruit fillings prepared with dietary fibers (inulin and pectin). The fillings were prepared in laboratory conditions starting from apple puree (45% w/w) in a wide range of soluble solids (30-70 °Brix). A 2k regression modeling was applied to assess the common and individual effects of total soluble solids and dietary fiber content on heat-stability and quality characteristics of prepared fillings. The use of multiple response optimization tool revealed that fillings prepared with 40-50 °Brix and addition of 3.5-5% inulin and 0.9-1.1% pectin possess the highest heat stability and sensory properties.

Keywords: fruit filling, dietary fiber, heat-stability, bakery index

Introduction

Nowadays, insight into the crucial significance of diet on human health and wellness is increasing among consumers, regulatory organizations and the food industry (Milliron *et al*., 2012; Chaput *et al*., 2012).

Modern consumers are quite interested in fresh fruits and vegetables as natural sources of bioactive substances with strong antioxidant properties. However, a huge part of the global crop is processed into different fruit and vegetable preserves, leading to great losses in biologically active compounds (vitamins, polyphenols, macro- and microelements, etc.) from natural agri-food raw materials. Nevertheless, processed fruits and vegetables may represent a rich source of dietary fiber (both soluble and insoluble), which provides many health benefits. The beneficial properties of these products diminish with increasing the liquid part and minimizing the solid one. Thus, fruit puree, jams, fillings and nectars with pulp are considered more salubrious due to the high amount of soluble fiber (pectin, protopectin, etc.) and polyphenolic fractions than the products manufactured with less solid part, such as clarified juices (Oszmiański *et al*., 2008).

For modern confectionery and bakery industry, the most important fruit preservers are fillings with heat-stable characteristics, which besides many technological advantages also possess health benefits associated with dietary fiber. Moreover, supplemental addition of dietary fibers into the composition of fruit fillings for providing better heat-stability, pumpability and absence of fruit separation, may also increase the beneficial health properties of the final product.

Certainly, pectin represents one of the main food ingredients found to give bake-stable functionality in various applications, including fruit fillings (Endreß *et al*., 1992; May, 2000). However, very often it requires the presence of another stabilizer with strong water-binding and thickening properties, because fruit compositions based just on pectin possess the tendency to syneresis depending on the total soluble solids content, pH and fruit type (Wang, 2009).

Generally, pectins are classified according to their degree of esterification (DE), which represents the amount of methylated galacturonic acid units on the pectin chain. High methoxyl pectins, with a DE of 55-80%, gel at low pH in the presence of large concentrations of sucrose or similar co-solutes. These pectins are dedicated to traditional high-sugar jam applications, because they provide the desired firm and stable texture to this type of fruit preparations. However, high methoxyl pectins are not always suitable for manufacturing heat-stable fruit fillings, because they may rapidly form thermally irreversible gels in the presence of sufficient sugars at low pH (Pranati & Rishabha, 2011), which can be further damaged during processing (pumping, mixing, injecting, etc.). As soon as the gel structure is broken down, the product is not heat-stable anymore. In contrast, low methoxyl pectins, with a DE of 20-50%, are quite insensitive and gel over a wide range of pH and sugar concentrations. Very often these pectins require calcium to gel. The latest commercial low-methoxyl pectins possess a high calcium tolerance. Thus, they have a positive influence on gelation so that less calcium is required, and enable the production of more elastic and transparent gels. They may be therefore used in heat-stable fruit filling applications (Lopes da Silva and Rao, 2006).

A lot of different interactions between pectin and other food biopolymers, such as pectin-gellan (Al-Ruqaie *et al*., 1997), pectin-alginate (Walkenstrom *et al*., 2003), and pectin-starch (Evageliou *et al*., 2000), have been largely studied in food systems. However, few studies describe the interaction between inulin and pectin.

From a technological point of view, inulin may be used along with pectin for preventing water release, because this dietary fiber possesses good water-binding capacity (Dysseler and Hoffen, 1995) and may serve as a bulking agent in fruit fillings (Ronkart *et al*., 2009). Its application may help to eliminate water migration from a fruit filling to the dough inside bakery products. Generally, it may be also achieved by equilibrating water content in two phases, which requires increasing the total soluble solids content or adding bulking agents (Wang, 2009).

Another important benefit of adding inulin and pectin into a fruit filling composition consists in their prebiotic properties which may regulate the composition of colonic microflora (Rao, 2001; Losada and Olleros, 2002; Manning and Gibson, 2004). In addition, these dietary fibers may significantly improve sensory characteristics, upgrading both taste and mouth-feel in many food formulations (Wang, 2009). Thus, addition of inulin and pectin into fruit fillings would assist to increase the daily intake of dietary fiber with prebiotic properties, while providing health and nutritional benefits to modern consumers.

Inulin has been increasingly used as a prebiotic food ingredient which possesses the ability to stimulate *in vivo* the growth of bifidobacteria at the expense of potential pathogenic microorganisms (Bohm *et al.*, 2005). It is composed of linear chains of D-fructofuranose molecules linked by b-2,1-glycosidic bonds terminating with a D-glucose fraction linked to fructose by a-1,2 bonds as in sucrose (Haraguchi *et al*., 2003). Given the special structure of inulin, it can produce high-purity fructo-oligosaccharides (FOS) by degradation, which also possesses prebiotic characteristics (Vervoort *et al*., 1998). According to *in vitro* tests carried out by Hotchkiss *et al.* (2003), pectin also acts as a prebiotic, preventing pathogens from binding to the intestine and enhancing the grows of probiotic bacteria in the large intestine, which stimulates gut health.

In order to act as a functional food ingredient, the prebiotic, however, should be chemically stable to various processing treatments such as elevated temperature, low pH, and Maillard reaction conditions. Once degraded to its component mono- and disaccharides, the prebiotic would no longer provide selective stimulation of beneficial microorganisms (Wang, 2009). Therefore, a long-chain chicory inulin was selected to develop heat-stable fruit fillings because it is more stable than a short-chain one obtained from *Jerusalem artichoke*. Moreover, according to our previous studies, only 10-20% of initially added inulin is destroyed under high temperature and low pH conditions during thermal processing (Cropotova, 2013). It was established by high performance liquid chromatography and further confirmed by validation colorimetric experiments that 80-90% of the initially added long-chain chicory inulin remains in fruit fillings after processing, which may claim the prebiotic properties of the finished product (Cropotova, 2013). Also, a fluorescence microscopy confirmed the presence of inulin particles (partially damaged due to thermal treatment) in differently formulated apple fillings (Cropotova *et al*., 2015a).

Measurement of color modification in fruit preserves represents a complex subject since it depends on consumer appreciation (Iguala *et al*., 2014). Unfortunately, heat processing of fruit fillings may lead to non-enzymatic browning reactions (Simsek *et al*., 2007) that impair sensory attributes of the product, especially due to color modification. For this reason, it is quite essential not only to conduct a sensory analysis of fruit fillings, but also to establish the possible relationships between the non-enzymatic browning index obtained by instrumental measurements and the acceptability of the color. Color represents one of the main sensory attributes of fruit preserves, since it can be directly estimated by the consumer (Quintas *et al*., 2007). Moreover, it may provide a rapid non-invasive tool to assess the extension of non-enzymatic browning reactions in the product, since they contribute to a specific coloration (Francis, 1995). Product color may be estimated either by a sensory panel or using analytical instrumentation (Quintas *et al.*, 2007). For example, besides organoleptic analysis, formation of brown pigments in fruit fillings during processing may be expressed through non-enzymatic browning index (NEBI) comprising the measurement of the product's absorbance at 420 nm (Cropotova *et al*., 2015b).

The aim of the present work was to study the influence of inulin and pectin on the heat-stable, physicochemical and sensory characteristics of fruit fillings prepared in a wide range of the total soluble solids – from 30 to 70 °Brix on the basis of experimental design technique.

Materials and methods

Raw material and ingredients

A commercial pasteurized apple puree (14 °Brix, "Orhei-Vit" LTD, Republic of Moldova) was used as fruit-based raw material for preparation of heat-stable fillings. The ingredients used in the study were: white sugar (moisture content 5.0%, sucrose content 99.85% d.m., JV "Südzucker Moldova", Republic of Moldova), long-chain inulin Orafti HP (moisture content 5.0%, Beneo, Belgium), low-methoxyl pectin GRINDSTED SF 580 (moisture content 12%, Danisco, Danemark) and citric acid ("EcoChimie" LTD, Chisinau, Republic of Moldova).

Chemicals

Ethyl alcohol (96% v/v) was purchased from "EcoChimie" LTD, Chisinau, Republic of Moldova.

Preparation of apple fillings

Preparation of apple fillings was carried out in laboratory conditions under atmospheric pressure from apple puree (45% w/w), sugar, inulin, pectin and citric acid. The filling formulations were made up of 30, 50 and 70 °Brix in the final product according to the planned experiment (Table 1) with different amounts of dietary fibers (pectin and inulin), as follows. Sugar was initially blended with apple puree and heated up to complete dissolution. The obtained homogeneous apple-sugar mixture served as the basis for all apple filling formulations presented in the study. The amount of sugar in the formulations varied from 16.57% w/w to 56.02% w/w depending on the final total soluble solids content of the fillings according to the planned design of experiments displayed in Table 1. Afterwards, inulin and pectin were dissolved in warm distilled water (60ºC) under continuous mixing and then simultaneously introduced into the previously prepared apple-sugar mixture. From a technological point of view, the low-methoxyl pectin GRINDSTED SF 530 with a high calcium tolerance is recommended for soluble solids of 45-70 ºBrix, while the long-chain inulin Orafti HP has very good syneresis control and thickening properties in low-sugar fruit compositions (<45 ºBrix) (Dysseler and

Hoffen, 1995;Vervoort *et al.*, 1998; Ronkart *et al.*, 2009), which together may act in synergism to create high-quality fruit filling compositions in the wide soluble solids content. After homogenization under intense blending, the prepared solution of citric acid (50% w/w) was added in amount of 0.6%. The total soluble solids content was measured by an ABBE benchtop refractometer (ABBE, Germany) and the filling composition was boiled to reach the required ºBrix according to the planned experiment (Table 1). The prepared fillings were put immediately into glass jars type III-58-150, sterilized at 100ºC for 15 minutes and stored at room temperature (20±2°C) for two days before analysis.

Control samples of apple fillings were prepared with 30, 50 and 70 °Brix without addition of inulin and pectin, according to the procedure described above.

The batch size comprised 60 experimental and 9 control samples of apple fillings, with a total of 3 replicates for each formulation.

Table 1. Experimental design matrix for heat-stable filling development

Sample	X_1 – inulin content (%)		X_2– pectin content (%)		X_4– total soluble solids (°Brix)	
	Coded values	Enoded values	Coded values	Enoded values	Coded values	Enoded values
1	-1	2.0	-1	0.7	-1	30
2	1	5.0	1	1.1	-1	30
3	-1	2.0	-1	0.7	1	70
4	1	5.0	1	1.1	1	70
5	-1	2.0	1	1.1	-1	30
6	1	5.0	-1	0.7	-1	30
7	-1	2.0	1	1.1	1	70
8	1	5.0	-1	0.7	1	70
9	0	3.5	0	0.9	0	50
10	0	3.5	-1	0.7	0	50
11	0	3.5	1	1.1	0	50
12	-1	2.0	0	0.9	0	50
13	1	5.0	0	0.9	0	50
14	-1	2.0	-1	0.7	0	50
15	1	5.0	1	1.1	0	50
16	1	5.0	-1	0.7	0	50
17	0	3.5	0	0.9	1	70
18	0	3.5	0	0.9	-1	30
19	-1	2.0	0	0.9	1	70
20	1	5.0	0	0.9	-1	30

The chemical and sensory analyses of prepared fillings were carried out after two days of storage at room temperature (20±2°C) in order to ensure proper gel strength of the product structure. The chemical assays were carried out randomly in three replicates.

Physicochemical analysis

The physicochemical analyses of prepared apple fillings were carried out at the Scientific Research Institute of Horticulture and Food Technology of Moldova.

The total soluble solids in each apple filling were measured in ºBrix at 20ºC using a standard Abbe refractometer. The ºBrix was read directly from the scale according to AOAC method 932.12 (AOAC, 2000). Dry matter content of all samples was determined according to AOAC method 934.06 (AOAC, 2000) by drying in a vacuum oven at 70 °C. The titratable acidity was determined according to AOAC method 942.15 (AOAC, 2000) by titration with a standard solution of sodium hydroxide (0.1N) to a faint pink end point after adding approximately 1 ml of phenolphthalein and calculated as citric acid.

The pH was determined through potentiometric method, introducing the electrode directly into the analyzed apple fillings.

The water activity of apple fillings was measured by using a portable *Novasina water activity* analyzer type ms1 at a temperature of 25ºC.

The non-enzymatic browning index (NEBI) of prepared apple fillings prior to baking was determined according to the method described by Meydav *et al*. (1977) with some modifications, as follows: ethyl alcohol in amount of 5 mL was added to 5 mL of an apple filling sample, centrifuged at 5,000 rpm for 10 minutes (Sigma 2-16 Benchtop Laboratory Centrifuge, Germany), and then absorbance of the obtained supernatant was read at 420 nm by using double-beam spectrophotometer SPECORD 210 (Analytik Jena, Germany, Germany). The obtained value was taken as the non-enzymatic browning index.

The relative bake stability of apple fillings was evaluated according to the method described by Young *et al*. (2003) with some modifications, as follows: a specific amount of a prepared apple filling was given into a base of special filter paper named “Blue ribbon” with a diameter of 120 mm by a metal ring with defined geometry (50 mm diameter and 10 mm height) and then baked under exactly fixed conditions: at a temperature of 200°C for 10 minutes.

The relative bakery index was determined by measuring the sample diameter before and after baking by placing a line across the sample according to the following formula:

$$BI(\%) = 100 - (D_2 - D_1)/D_2 \cdot 100 \quad (1)$$

where BI is the relative bakery index (%), D_1 is the average sample diameter before baking (mm), D_2 is the average sample diameter after baking (mm).

Depending on the shape of the sample diameter, two to four lines were drawn, and the average was calculated.

Each of the tests was made in triplicate and all experimental measurements were expressed as average of conducted analyses ± standard deviation.

Sensory analysis

A panel of 15 tasters, 7 men and 8 women, performed a sensory analysis of experimental and control samples of apple fillings. The experimental samples of apple fillings containing different amounts of inulin and pectin were compared to three control samples of apple fillings prepared with 30, 50 and 70 °Brix from the same apple puree without adding inulin and pectin regarding sensory acceptability. The samples were served at room temperature in disposable, transparent, odor-free plastic cups marked with three random digit numbers. Each panelist was provided with approximately the same quantity of each apple filling sample and mineral water for rinsing the mouth. The equal amounts of the analyzed apple fillings were portioned in each plastic cup. The trays with plastic cups containing the filling samples and questionnaires were prepared in advance and randomized to make sure that the order of the samples would not affect the final result.

The acceptability of the color, flavor, taste, consistency and the overall acceptability were assessed by using a hedonic scale of nine points (1 - disliked extremely; 5 - neither liked nor disliked; 9 - liked extremely) (Meilgaard *et al.*, 1999).

Experimental design and statistical analysis

According to the hypothesis of the study, the heat-stable properties of fruit fillings are mainly influenced by the total soluble solids content and percentage of dietary fibers (inulin and pectin), while other parameters remain constant (amount of fruit part, pH and processing conditions). Therefore, the following experimental factors were investigated in two-level factorial design with added central point: amount of added inulin (2.0, 3.5 and 5.0%), pectin (0.7, 0.9 and 1.1%) and the total soluble solids content (30, 50 and 70 °Brix). The twenty combinations of three independent factors according to the design of experiments displayed in Table 1 were run in triplicate. The values of the experimental factors were chosen in a base of preliminary experimental results. The heat-stability of fruit fillings as a response variable was expressed through the relative bakery index (BI). All experiments adjusted by the design planned in coded and encoded form of process variables, were conducted randomly.

The statistical adequacy of the regression equations derived in the study was verified by multivariate analysis of variance (MANOVA), using Statgraphics Centurion Software XVI (Statistical Graphics Corporation, USA). 3D surface plots of obtained polynomial equations were drawn by using MATHCAD v.15.

Results and discussions

Physicochemical parameters

The main physicochemical and sensory parameters of the apple fillings prepared with inulin and pectin according to the planned experiment (Table 1) are displayed in Table 2, while those of the control samples are shown in Table 3. The formulated apple fillings (both experimental and control samples) were prepared with 30, 50 and 70 °Brix and their titratable acidity varied from 0.48 to 0.61 g/L citric acid.

Water activity values ranged between 0.701 and 0.941 depending on the dry matter of apple fillings; the higher values of water activity were observed for control samples of apple fillings prepared without addition of inulin and pectin. The change of non-enzymatic browning index in apple fillings had a dynamic character associated with certain color modifications having place during thermal treatment. The experimental and control samples of apple fillings were prepared similarly; however the non-enzymatic browning index values ranged even between the samples with the same total soluble solids content and pH (Table 2 and 3). This suggests that this parameter is also influenced by the addition of inulin and pectin to filling formulations and their degradation during heat treatment.

As expected, experimental samples of the fillings prepared with inulin and pectin showed significantly higher heat-stable properties than the control ones (Table 2 and 3).

Table 2. Physicochemical and sensory characteristics of control samples of apple fillings

№	TSS, °Brix	Physicochemical characteristics					Sensory attributes				
		Dry matter, %	a_w	TA, g/L	BI, %	NEBI	Color	Flavor	Taste	Consistency	Overall acceptability
1	30	32.89 ±0.11	0.94 ±0.01	0.58 ±0.02	45.27 ±0.25	1.48 ±0.19	8.02 ±0.32	7.80 ±0.25	7.80 ±0.17	8.65 ±0.12	7.90 ±0.11
2	50	53.52 ±0.11	0.85 ±0.02	0,48 ±0.02	62.43 ±0.40	2.29 ±0.09	7.17 ±0.54	6.70 ±0.39	7.00 ±0.52	8.00 ±0.42	7.00 ±0.06
3	70	72.31 ±0.27	0.75 ±0.01	0.48 ±0.02	72.40 ±0.36	3.12 ±0.11	6.29 ±0.21	6.00 ±0.67	6.05 ±0.81	7.20 ±0.68	6.40 ±0.22

*Data are expressed as mean ± standard deviation ($p<0.05$)
TSS – total soluble solids, a_w – water activity, TA – titratable acidity (calculated as citric acid), BI – relative bakery index, NEBI – non-enzymatic browning index.

After processing the experimental data of the bakery index presented in Table 3, the following regression equation was obtained, describing the common effect of the total soluble solids and ratio of pectin and inulin on the heat-stable characteristics of fruit fillings in terms of actual values:

$$BI_f = 39.136 \cdot I + 114.922 \cdot P - 4.347 \cdot TSS - 0.464 \cdot I \cdot P \cdot TSS - 107.701 \cdot I \cdot P + \\ + 0.519 \cdot I \cdot TSS + 8.045 \cdot P \cdot TSS + 65.355 \cdot I \cdot P^2 - 0.033 \cdot TSS^2 \cdot P^2 - 119.388 \cdot P^3 \quad (2)$$

where BI_f is the relative bakery index of the apple fillings (%), I is the added inulin content (%), P is the added pectin content (%) and TSS is the total soluble solids (°Brix).

Table 3. Quality characteristics of apple filling samples from experimental design

No	Physicochemical characteristics					Sensory attributes				
	Dry matter, %	a_w	TA, g/L	BI, %	NEBI	Color	Flavor	Taste	Consistency	OA
1	33.53 ±0.11*	0.92 ±0.01	0.59 ±0.02	67.67 ±0.58	1.71 ±0.44	8.09 ±0.17	8.01 ±0.98	8.08 ±0.61	8.81 ±0.11	8.32 ±0.21
2	37.32 ±0.15	0.90 ±0.03	0.61 ±0.01	66.67 ±0.58	2.07 ±0.01	8.08 ±0.18	8.29 ±0.53	8.61 ±0.13	8.97 ±0.02	8.82 ±0.11
3	73.17 ±0.27	0.73 ±0.01	0.48 ±0.02	68.02 ±0.58	3.35 ±0.09	6.29 ±0.86	6.00 ±0.17	6.32 ±0.44	7.42 ±0.59	6.42 ±0.98
4	76.38 ±0.18	0.69 ±0.02	0.51 ±0.03	88.65 ±0.01	3.71 ±0.07	6.27 ±0.74	6.25 ±0.61	6.85 ±0.25	7.56 ±0.11	6.91 ±0.21
5	34.83 ±0.31	0.92 ±0.02	0.60 ±0.01	66.65 ±0.58	1.71 ±0.11	8.09 ±0.14	8.12 ±0.63	8.13 ±0.37	8.87 ±0.05	8.45 ±0.26
6	36.75 ±0.09	0.92 ±0.01	0.61 ±0.01	71.40 ±0.36	2.07 ±0.02	8.07 ±0.18	8.20 ±0.34	8.56 ±0.21	8.91 ±0.04	8.71 ±0.27
7	74.29 ±0.12	0.73 ±0.01	0.48 ±0.02	87.75 ±0.01	3.36 ±0.02	6.28 ±0.67	6.09 ±0.24	6.37 ±0.13	7.46 ±0.17	6.54 ±0.38
8	76.13 ±0.36	0.70 ±0.03	0.49 ±0.01	97.02 ±0.01	3,71 ±0.27	6.27 ±0.09	6.16 ±0.31	6.81 ±0.22	7.52 ±0.31	6.78 ±0.82
9	56.81 ±0.10	0.83 ±0.02	0.48 ±0.02	94.98 ±0.01	2.71 ±0.17	7.18 ±0.17	7.15 ±0.56	7.47 ±0.42	8.18 ±0.11	7.62 ±0.71
10	55.62 ±0.13	0.84 ±0.01	0.55 ±0.03	82.47 ±0.25	2.71 ±0.12	7.17 ±0.35	7.11 ±0.21	7.44 ±0.89	8.12 ±0.93	7.56 ±0.17
11	56.98 ±0.21	0.83 ±0.02	0.56 ±0.02	93.41 ±0.43	2.71 ±0.25	7.18 ±0.24	7.20 ±0.46	7.49 ±0.18	8.21 ±0.78	7.68 ±0.75
12	55.12 ±0.23	0.84 ±0.03	0.55 ±0.01	94.67 ±0.58	2.53 ±0.27	7.19 ±0.12	7.07 ±0.38	7.22 ±0.22	8.12 ±0.77	7.44 ±0.81
13	57.15 ±0.16	0.83 ±0.02	0.55 ±0.02	95.32 ±0.01	2.89 ±0.15	7.18 ±0.19	7.23 ±0.24	7.71 ±0.57	8.22 ±0.73	7.81 ±0.48
14	53.37 ±0.11	0.84 ±0.02	0,55 ±0.03	74.25 ±0.36	2.53 ±0.08	7.19 ±0.16	7.03 ±0.65	7.20 ±0.13	8.11 ±0.27	7.37 ±0.75
15	57.35 ±0.28	0.82 ±0.001	0.56 ±0.02	93.75 ±0.01	2.89 ±0.22	7.18 ±0.32	7.27 ±0.49	7.73 ±0.11	8.25 ±0.16	7.87 ±0.27
16	57.13 ±0.34	0.83 ±0.03	0,55 ±0.01	96.98 ±0.01	2.88 ±0.17	7.17 ±0.48	7.18 ±0.54	7.68 ±0.31	8.21 ±0.16	7.74 ±0.21
17	75.42 ±0.12	0.71 ±0.01	0.49 ±0.02	95.80 ±0.01	3,53 ±0.25	6.28± 0.77	6.13 ±0.51	6.58 ±0.49	7.48 ±0.64	6.68 ±0.25
18	35.28 ±0.25	0.92 ±0.02	0.60 ±0.01	72.76 ±0.36	1.89 ±0.12	8.08 ±0.18	8.17 ±0.51	8.35 ±0.56	8.89 ±0.07	8.58 ±0.29

19	73.33 ±0.32	0.73 ±0.03	0.48 ±0.02	92.44 ±0.01	3.35 ±0.22	6.29 ±0.52	6.05 ±0.55	6.34 ±0.80	7.44 ±0.67	6.47 ±0.91
20	36.15 ±0.36	0.91 ±0.01	0.61 ±0.03	70.06 ±0.58	2.06 ±0.11	8.08 ±0.53	8.25 ±0.28	8.59 ±0.32	8.93 ±0.07	8.76 ±0.24

*Data are expressed as mean ± standard deviation ($p<0.05$)
a_w – water activity, TA – titratable acidity (calculated as citric acid), BI – relative bakery index, NEBI – non-enzymatic browning index, OA – overall acceptability.

The model presented above may be successfully used to predict the heat-stable properties of fruit fillings prepared with a mixture of inulin and pectin within the limits of the experimental factors.

Typical 3D surface plots, displaying the influence of added inulin and pectin on the bakery index of fruit fillings for 30-45 °Brix and 50-70 °Brix respectively, are shown in Fig. 1 (a and b).

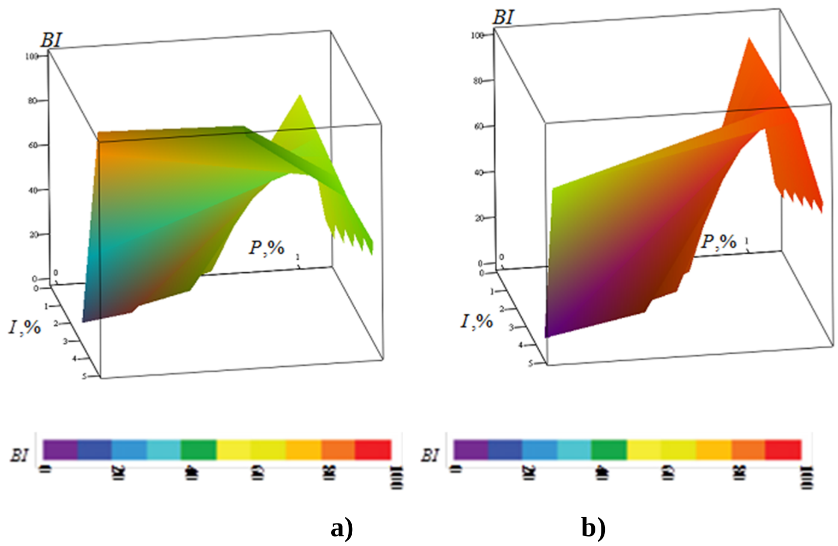

Figure 1. 3D surface plots displaying the influence of added inulin and pectin amounts on bakery index of fruit fillings prepared with: a) 30-45 °Brix; b) 50-70 °Brix

The response surface plots of the polynomial equation represented above have been plotted using MATHCAD v.15 as a function of two independent variables, i.e. the percentage of inulin and pectin, while the third independent variable (the total soluble solids content) remained constant (Figure 1) in order to visualize their common effect on the response variable – the relative bakery index.

Judging from the model, it is obvious that pectin mainly influences the bakery index of the fruit fillings; however, high doses of inulin also help to increase its value, while bakery-stable properties are more expressed from 50 to 70 ºBrix. It may be explained due to the fact that the addition of simple sugars generally modifies polysaccharide gel properties, by establishing junction zones and rising melting temperature (Meilgaard *et al*., 1999). Another explanation is that, in food systems containing pectin, generally high sugar concentrations (50-70%) stabilize the junction zones within the hydrocolloid gel network by a complex mixture of hydrogen bonds, hydrophobic and electrostatic interactions (Oakenfull, 2000). However, a sugar content above 70% may actually break down junction zones, resulting in less gel-like systems (Mitchell, 2000), which causes diminution of the bake-stable properties of fruit fillings.

According to the present research, it was also revealed that low-methoxyl pectin would be more advantageous to use in combination with long-chain inulin (by finding their optimal ratio that provides synergetic effect for each of the total soluble solids content) for manufacturing traditional fruit fillings in order to enhance heat-stable, sensory and textural characteristics of the final product, while providing health benefits due to the prebiotic properties of the proposed ingredients.

Sensory data

The results of the sensory evaluation of apple fillings indicated that all samples of the product were acceptable to consumers. However, fillings with 30 ºBrix had the highest mean scores for all attributes being compared (numbers 1, 2, 5, 6, 18 and 20). The majority of panelists scored the tested fillings higher for taste and consistency. Probably, this is due to the fact that the low methoxyl pectin used in the preparation of apple fillings gives these products a soft and smooth texture (Lopes da Silva and Rao, 2006). The color and flavor of all filling samples had the lowest scores compared with other sensory parameters.

Moreover, the hedonic test revealed that all apple filling samples with addition of inulin and pectin had color, flavor, taste, consistency and overall acceptability scores comparable or above those of control samples (Tables 2 and 3). However, the level of addition of inulin and pectin influenced differently the sensory attributes of experimental samples of apple fillings evaluated in the study.

The following predictive equations describing the influence of inulin, pectin and the total soluble solids on each of sensory attributes were derived using experimental design methodology:

$$C_f = 9.445 - 0.004 \cdot I - 0.045 \cdot TSS \quad (3)$$

$$F_f = 9.321 + 0.052 \cdot I + 0.217 \cdot P - 0.051 \cdot TSS \quad (4)$$

$$T_f = 8.994 + 0.162 \cdot I + 0.117 \cdot P - 0.044 \cdot TSS \quad (5)$$

$$Con_f = 9.705 + 0.032 \cdot I + 0.124 \cdot P - 0.035 \cdot TSS \quad (6)$$

$$O_f = 9.314 + 0.124 \cdot I + 0.295 \cdot P - 0.048 \cdot TSS \quad (7)$$

where C_f is the color score, F_f is the flavor score, and T_f is the taste score, Con_f is the consistency score, O_f is the overall acceptability of apple fillings, I is the added inulin content (%), *P* is the added pectin content (%) and *TSS* is the total soluble solids (°Brix).

Color scores (Tables 2 and 3) revealed that both control and experimental samples with less Brix degrees were perceived as having significantly more desirable color than those with 70 °Brix. Probably, this is due to the negative effect of thermal processing leading to the destructive changes in sensory quality through non-enzymatic browning, including the Maillard reactions, which involve the oxidation of ascorbic acid and transformations of sugars in the product (Simsek, 2007). However, the influence of inulin on the color perception of experimental samples of apple fillings is very small, while pectin does not affect this sensory parameter at all (Eq. 3). According to the hypothesis of the study, during thermal processing long-chain inulin undergoes partial hydrolysis followed by formation of fructooligosaccharides, which may further decompose into monomeric fructose units in acidic environment under high temperatures (Iguala, 2014). The reactions of the acid hydrolysis of inulin are accompanied by additional formation of undesirably yellow colored hydrolysate and fructose anhydride (Kim and Lim, 2002), which may significantly affect the color perception of the final product.

Since apple fillings contain both sugar and amino acids, non-enzymatic browning, including Maillard reactions, are the main causes of color degradation in these types of products (Cropotova *et al*., 2015b). The time and temperature of thermal processing are the primary factors affecting non-enzymatic browning reactions in fruit preserves. In order to minimize the production of browning compounds, heat treatment should be kept as short as possible. A short-time thermal treatment at 80-100°C is usually sufficient to inactivate any present microorganisms and undesired enzymes (Figuerola, 2007). However, non-enzymatic browning may take place at temperatures below 100°C and contribute to color changes during storage since this reaction also results in the formation of HMF (Aslanovaa *et al*., 2010; Cropotova *et al*., 2015b).

Probably, the color assessment may also depend on non-enzymatic browning taking place in apple fillings during processing and sterilization; therefore, the following inter-correlation between color scores and non-enzymatic browning index of apple fillings may be pointed out:

$$C_f = 9.04 - 0.88 \cdot NEBI_f \tag{8}$$

where C_f is the color score and $NEBI_f$ is the non-enzymatic browning index of apple fillings.

The linear regression displayed above (Eq. 8) showed a significant correlation between non-enzymatic browning index and color scores ($R^2 = 0.95$, $P<0.01$) in differently formulated apple fillings.

Despite the affirmations of many authors, pointing to an increase in flavor with addition of inulin in food products (Tárrega *et al*., 2010; Arcia *et al*., 2011), this sensory parameter was not affected by addition of inulin in apple fillings prepared with inulin and pectin in comparison with control samples. According to the regression equation describing the influence of inulin, pectin and the total soluble solids on fruit fillings' flavor (Eq. 4), inulin possess the smallest positive effect on flavor perception, while pectin enhances and the total soluble solids reduce the assessment of this sensory characteristic.

The joint addition of inulin and pectin to apple fillings led to an increase in taste, consistency and overall acceptability, which was greatest in samples with less Brix degrees (Table 2). Likewise, the samples containing inulin and pectin were clearly preferred to the control ones without inulin-pectin mixtures (Table 3).

According to the regression equations displaying the effect of added dietary fibers and the total soluble solids on sensory attributes (Eqs. 5-7), inulin and pectin improved the organoleptic perception of taste, consistency and overall acceptability of apple fillings, while the increase in the total soluble solids impairs the sensory profile. The derived equations 3-7 can be used to predict the sensory characteristics of apple fillings prepared within the limits of the experimental factors.

Optimization of formulations of fruit fillings

Conducting a product optimization permits to find the best formulations based on a combination of independent factor levels that simultaneously satisfy the requirements established for each of the response variables. In the current research, optimal formulations were predicted by the multiple response optimization design (Myers, 2009) conducted with Statgraphics Centurion XVI to optimize formulations of fruit fillings on the basis of three desirable responses (bakery index, overall acceptability and non-enzymatic browning index).

In order to realize simultaneous optimization, the most important dependent variables were maximized or minimized depending on their positive or negative influence on the final product quality. Thus, the desirable responses describing the bakery index and overall acceptability of fruit fillings were set at their higher levels (90-100% and 7.5-9.0 points respectively), while for the single undesirable response variable indicating non-enzymatic browning index a lower level of 1-3 was established in the optimization design (Table 4).

Table 4. Responses variables and criteria of analysis for multiple response optimization

Responses	Predicted values		Ratio (in %)
	Highest	Lowest	(1-L/H)·100%
BI*	100	90	10.00
O_f	9.0	7.5	16.67
NEBI	3.0	1.0	66.67

* BI – relative bakery index, O_f – overall acceptability of apple fillings, NEBI – non-enzymatic browning index.

The variation between the lowest and highest predicted values of the selected response variables was ≥ 10% (Table 4). Therefore, the predicted values of the multiple response optimization design were found significantly relevant to optimize the experimental conditions for formulating heat-stable fruit fillings.

In order to visualize the process of multiple response optimization, a typical 3D optimization plot (displayed in Fig. 2) was built with MATHCAD v.15. It helps to assess the simultaneous influence of inulin, pectin and total soluble solids content on the bakery index, overall acceptability and non-enzymatic browning degree of fruit fillings and to select visually the best region of independent factors for formulating high-quality product.

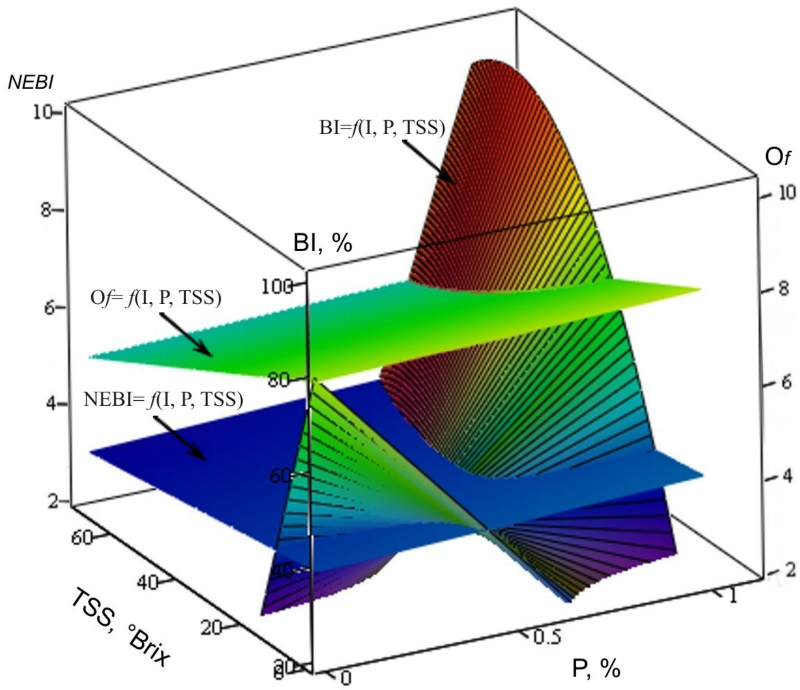

Figure 2. 3D optimization plot for formulating high-quality heat-stable fruit fillings

According to multiple response optimization results, the best model formulation of fruit filling predicted by the Statgraphics Centurion Software XVI should contain 40-50 °Brix, and be prepared with addition of 3.5-5.0% inulin and 0.9-1.1% pectin.

Conclusions

The study revealed that the simultaneous use of inulin and pectin not only enhance the heat-stable properties of fruit fillings, but, besides providing certain nutritional advantages, may also improve the sensory characteristics of the product. Sensory properties varied among the different experimental apple filling samples prepared with inulin-pectin mixtures, but in all cases they were enhanced comparing to the control ones. There were derived statistically adequate regression equations in terms of actual factors describing the common effect of amount of the added dietary fibers and the total soluble solids on the heat-stable and sensory properties of apple fillings.

The optimization tool was applied to predict fruit fillings containing inulin and pectin with the highest heat-stable and sensory properties. Thus, according to multiple response optimization results, the fruit fillings having the highest heat-stable and organoleptic characteristics should contain 40-50 °Brix, and be prepared with addition of 3.5-5.0% inulin and 0.9-1.1% pectin.

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