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Research Article

Production of natural bitter orange (*Bitter orange aurantium*) sauce

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Abstract

Sour sauces are an important part of Turkish cuisine and the Mediterranean diet. They are generally used as a flavoring in salads, cold appetizers and various dishes such as stuffed meatballs, raw meatballs and casseroles. In this study, it was aimed to obtain a sauce with a high consistency from the juice of bitter oranges grown in the Mediterranean climate, which is not evaluated due to its bitter taste due to its high naringin content and to be used in salads or meals. Carob molasses pulp (CMP) was used in the sauce preparation process to obtain high consistency. In addition to providing high consistency, CMP has increased the nutritional value of the sauce. For this purpose, bitter orange (Bitter orange aurantium) juice was evaporated in the open boiler until it reached 25-30 ^{0}Brix. After adding 2% CMP to the semi-evaporated bitter orange juice, the evaporation process was continued till it reached the final concentration of 55 ^{0}Brix. The obtained sauce was evaluated in pH, total phenolic content, total antioxidant activity, color and sensory properties. As a result, the total phenolics content was 785±4.00 mg GAE/L and the total antioxidant capacity was 77±0.00 µmol TE/L. The results showed that the citrus sauce supplemented with CMP was exposed to less heat treatment and lower change in its lightness according to the control and it was liked more than the control in terms of color, consistency, taste and overall acceptability. Consequently, a new high-consistency sour bitter orange sauce was produced which was appreciated by consumers.

INTRODUCTION

Sours are important in Turkish cookery and the Mediterranean diet (1). They are generally used as a flavorer in salads, cold appetizers, and various dishes such as stuffed meat, raw meatballs and casseroles (2). They present products with a high added value and are also quite readily manufactured (3). There are various indigenous sour species due to cultural characteristics and regional differences, and they are mostly made and consumed with traditional methods (1).

To produce sours, traditional and typical methods have been used. In a traditional method, a juice is concentrated by boiling, until it reaches certain consistency in an open boiler. In a typical one, it is evaporated in either an open boiler or under a vacuum, although a different type of stuff requires different production methods. When sour is concentrated, its nutritional properties increase (4). But 5-Hydroxymethyl furfural (HMF) which causes browning is occurred from the reaction between glucose and amino acid, particularly under acidic conditions (5, 6). So it is a substantial quality parameter for concentrated food products, and it indicates the degree of heating (4). It mainly causes color changes, loss of sugar and vitamin C that affect product quality (4, 7). Also, it is harmful to health from negative effects on human beings such as carcinogenic, genotoxic, organotoxic etc. (8). On this basis made works shows that HMF is taken under control and the quality characteristics of the product are preserved when the production is done under a vacuum with lower temperature and shorter heat treatment conditions in an industrial (9).

Turkish cuisine become prominent with its eating and drinking cultures similar to world cuisines such as Italian, Chinese, French and Indian cuisines and is among the cuisines with the most valuable and regional product variety in the world. For example pomegranate sour, black mulberry sour, plum sour, sumac sour, lemon sour, bitter orange juice and verjuice are consumed in the Southeastern Anatolia region and east Mediterranean regions (10) and apple sour is consumed in Kastamonu (11). Generally, they are produced by traditional methods and contribute to the family economy (10). Among these pomegranate is the most well-known and widely consumed. It could be consumed as fresh fruit, fruit juice, fruit juice concentrate, marmalade, wine or liquor and especially sour or sauce. Although sour is produced by traditional methods that change according to regions and also it is produced on an industrial scale (12, 13). Bitter orange fruit is the most limited consumed because limonin and naringin compounds in the fruit cause an unpleasant bitterness (14). So, it is generally not consumed as fresh fruit or fruit juice. It is generally used in the production of jam, marmalade, essential oil and pectin and sour is produced from bitter orange juice by traditional methods on small scales. Bitter orange has high antioxidant properties due to the content of limonin and naringin and it also contains important food ingredients such as niacin, folic acid, dietary fiber, pectin, potassium, calcium and magnesium. But which has important effects in terms of health and is grown abundantly in our country cannot be adequately evaluated (15).

While only the fruit is used in sour production, additives are added to change the flavor, aroma and consistency properties in sauce production (1). Thickeners affect food viscosity and texture (16). So they are added to sauces to provide certain consistency and meet sensory demands (17). Carboxymethyl cellulose (CMC), methylcellulose (MC), hydroxypropyl methylcellulose (HPMC) and gum arabic are the examples of the thickeners which are used in sauces (16). In

this point, dietary fibers can be a good alternative to increase consistency of the product naturally.

Dietary fibers use as a functional component lately besides playing an important role in the prevention of diseases such as heart disease, hypertension, diabetes, obesity and gastrointestinal disorders. There are increasing studies related to its industrial recovery and addition to food products (18, 19).

Carob fruits include a high amount of dietary fibers and are composed of sugars, polyphenols (e.g. tannins, flavonoids, phenolic acids), minerals (e.g. K, Ca, Mg, Na, Cu, Fe, Mn, Zn) and vitamins (e.g. E, D, C, Niacin, B6, and folic acid). Due to its antioxidant composition, and high content of fibers, it can protect humans from some diseases such as cancer and cardiovascular diseases (20, 21).

The most produced and consumed product of carob fruit is carob molasses. In molasses production, the remaining part after pressing is the carob molasses pulp which contains a high percentage of dietary fiber. So carob molasses pulp is an important raw material that should be valued because of increasing interest in the natural antioxidants which help protect against oxidative damage (22-24). But in Turkey, it is generally used as animal feed or waste. In scientific studies, there are studies in which carob molasses pulp is used as a food additive by Ozdemir et al. (25, 26).

In this study, it was aimed to produce a new bitter orange sauce that can be used in meals, especially in salads by consumers of citrus fruit, which is widely grown in the Mediterranean region but not sufficiently evaluated.

MATERIALS AND METHODS

Production of Carob Molasses Pulp

In this study, raw carob molasses pulp obtained from Atışeri company producing carob molasses in Mersin was used. Raw carob molasses pulp was dried in a laboratory oven (EN400, Nuve, Turkey) at between 50 ± 5 °C for 8 h. Then it was passed through sieves with a mesh diameter of 75 μm after grinding with a mill (Model AR 1056, Arzum, İstanbul, Turkey), and this was called carob molasses pulp (CMP).

Production of Bitter Orange (*Bitter Orange Aurantium*) Sauce

Bitter orange was collected from Mersin, Turkey. To provide a homogeneous sampling, the collected fruits were washed on the same day, cut in the middle and squeezed with the help of an extractor. It was passed through sieves with a mesh diameter of 120 μm to remove coarse fruit pulp particles and bitter orange juice was obtained. It was stored in a freezer (D70430N, Arçelik, Turkey) at -18 °C, until production. The steps given in fig. 1 were followed in the production of control (without CMP) and bitter orange sauce with CMP.

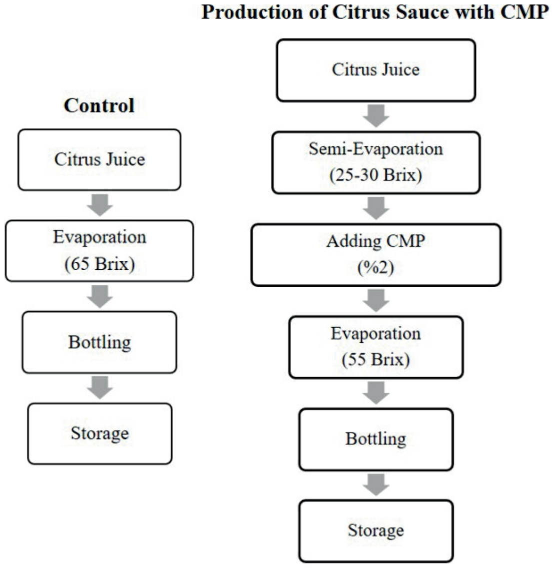

Figure 1. Schematic overview of the production

Physical and Chemical Analyzes

Total soluble solids (°Brix) of bitter orange juice, control and bitter orange sauce with CMP were directly determined at 20 °C with a digital refractometer (VBR90A, Soif, İstanbul, Turkey). Similarly, pH of samples was determined by a pH meter (ST300, Ohaus, USA) at 20°C.

Total phenolic content of control and bitter orange sauce with CMP was determined according to the Folin-Ciocalteu method described by Singleton et al. (27) with some modifications. A 0.5 ml of Folin-Ciocalteu reagent was added to 0.5 ml of diluted sample and mixed for 5 minutes. After adding 3 ml of 10% Na2CO3 solution, the volume was completed to 10 ml with distilled water and the sample mixture was kept in the dark for 30 minutes. Absorbance values of all samples were measured at 760 nm wavelength in a spectrophotometer (UV 1800, Rayleigh, Beijing, China). Gallic acid was used as a standard (0-500 ppm), and distilled water was used as a blank. Results were given as mg gallic acid equivalents (mg GAE/L sample). Three repetitions of the experiment were conducted.

Antioxidant activity of control and bitter orange sauce with CMP was determined according to the DPPH method described by Brands-William et al. (28) with some modifications. A 3.9

ml of DPPH (0.1 mmol) was added to the 0.1 ml diluted sample and mixed for 2 minutes. Then the sample mixture was kept in the dark for 30 minutes. Absorbance values of all samples were measured at 545 nm wavelength in a spectrophotometer (UV 1800, Rayleigh, Beijing, China) and Trolox was used as a standard (0.005-0.05 mmol) and 80% methanol was used as a blank.

Results were given as mmol Trolox/L sample. Three repetitions of the experiment were conducted.

Color Measurement

Color was determined with a colorimeter using illuminant D-65 with a 4° standard observer (PCE-CSM 1, PCE, Alicante, Spain). Mean L* (lightness), a* (redness-greenness) and b* (yellow-blueness) values of each sample were determined. The colorimeter was calibrated with white and black calibration plates.

Sensory Evaluation

Sensory attributes (color, consistency, odour, taste and overall acceptability (simple and with salad) of bitter orange sauce supplemented with CMP were assessed using a 5-point (1:extremely dislike to 5:extremely like) hedonic scale. For this purpose, sensory analyzes were carried out by 15 trained panelists consisting of Toros University staff. Unsalted crackers and water were offered to cleanse the palates between sample tasting.

Statistical Analysis

The experimental data were evaluated with a student-t test ($p < 0.05$) to determine significant differences between the means of the two groups which were control and bitter orange sauce supplemented with CMP using the analysis program SPSS (Version 25, IBM, USA).

RESULTS

pH and total soluble solids (°Brix) of the samples are given in Table 1. In general, sauces with a unit value of about 65 ^{0}Brix for similar sauces such as pomegranate syrup are used in meals. Hepsağ et al. (29) reported that Brix values ranged from 58.0% to 69.5% for pomegranate sauce and the mean value was around 63.41%. Uçan et al. (9) evaporated the bitter orange sour until it reaches 68.5 °Brix. However, in this study, boiling was applied up to 55 °Brix with the addition of CMP and the boiling time was 75 min. For this, after the concentration of bitter orange juice had been brought to 25-30 °Brix with 45 min semi-evaporation process in the first stage, CMP was added and in the second stage, a further evaporation process was applied up to 55 °Brix for 30 min. Thus, the evaporation process time was reduced by 20 min. compared to the control sauce process.

Polysaccharide hydrocolloids which are solutions or colloidal suspensions are Non-Newtonian fluids. Viscosity increases due to the change in shearing stress and thickening occur. The thickening effect of hydrocolloids is usually existed in the form of viscosity values of aqueous solutions (colloidal suspension), at identified conditions such as concentration, pH, temperature, and ionic strength. Polysaccharide hydrocolloids are preferred over proteins because they show more effective thickening ability even at low concentrations (30).

Ozdemir et al. (25) reported that the crude carob fiber (CCF) was 20% in carob molasses pulp on a dry basis. The total crude fiber was 79.23% in CCF flour and 52.03% of crude fiber

contains lignin. Total other insoluble fibers were calculated as a difference between the CCF flour's moisture, ash, protein and lignin contents. Therefore, other insoluble fibers which were predicted to be cellulose, hemicellulose and insoluble polyphenols 27.20%.

As can be seen from Table 1, pH values with and without CMP sauces were higher than bitter orange juice. On the other hand, the pH of bitter orange sour (control) was determined 2.07. Since the concentration of acidic components increased with the evaporation process, the pH values of the control (65 °Brix) and bitter orange sauce supplemented with CMP (55 °Brix) were lower according to bitter orange juice. Foods are classified according to their pH values as follows: highly acidic foods (pH<3.7), acidic foods (pH: 3.7-4.6), middle acidic foods (pH:4.6-5.3) and low acid foods (pH>5.3) (9). Gün (31) reported that the pH value of bitter orange juice is between 2.60 and 2.86. It was reported that the bitter orange was similar to lemon in terms of acid. Pathogenic microorganisms cannot grow in acidic conditions, so it is an important parameter for food safety (32).

The total polyphenol content (in terms of gallic acid) of the samples were given in Table 2. The sauce supplemented with CMP showed an increase in total phenolic contents and antioxidant activity. When the mean value of samples was compared statistically, while there was a significant ($p < 0.05$) difference between the total phenolic contents, there was no significant ($p > 0.05$) difference between the antioxidant activities.

As can be seen from the table, there are high amounts of polyphenolic components in the control sample. These components are associated with the increase in the concentration of flavonoids in bitter orange juice after evaporation. Gattuso et al. (33) reported that the level of flavanones such as naringin, neohesperidin and neoriocitrin in bitter orange juice (*C. aurantium* juice) was found 1.97, 0.87, 0.77 and 0.73 mg/100 mL, respectively. Although TPC was high in bitter orange sauce, TCA was low. Some bitter orange fruits have a bitter taste. This bitter taste is related to flavonoids (naringin, neohesperidin) and limonoids (limonin, nomilin). Naringin is a bitter-tasting flavanone glycoside found in grapefruit, pomelo and bitter orange. It has been reported that the amount of naringin decreases with the increase in fruit maturity (34). On the other hand, with the addition of CMP, an increase of 12% and 11%, respectively, was observed in both TPC and TCA. These increases were thought to come from the polyphenolic compounds in the structure of the added CPM.

Ozdemir et al. (26) reported that the total polyphenol content (in terms of GAE) of the flour of carob molasses pulp was 3.05% on a dry basis. There is a significant amount of phenolic compound in carob fruits. More specifically, gallic acid, flavonoids, syringic acid, quercetin, rutin, myricetin, catechin and epicatechin are available in the product. The total polyphenols in the carob pod ranged from 0.19 and 9.28 mg GAE/g. Phenolic compounds are beneficial to health due to their high antioxidant activity.

Ozdemir et al. (26) reported that the antioxidant activity of carob molasses pulp (CMP) flour was found to be 0.91% on a dry basis. It has been reported that the antioxidant activity of CMP flour was higher than carob pod, flour and pulp because of the existence of phenolic compounds in its content.

Color Measurement

The Hunter L*, a* and b* values of the samples were given in Table 3. Concentration was caused

Table 1. pH and total soluble solids (°Brix) values of the samples

Samples	Total Soluble Solids (°Brix)	pH
Bitter orange juice	8.67±0.58	2.51±0.13
Bitter orange sour (Control)	65±0.00	2.07±0.00
Bitter orange sauce with CMP	55±0.00	2.15±0.06

Table 2. Total phenolics content (TPC) and total antioxidant activity (TAC) values of the samples

Samples	TPC (mg GAE/L)	TAC µmol TE/L
Bitter orange sour (Control)	696±14.42^{a}	69±0.00^{a}
Bitter orange sauce with CMP	785±4.00^{b}	77±0.00^{b}

There is no difference between the averages in the same column and shown with the same letters p<0.05.

to the darkening of the samples with non-enzymatic browning reactions (35). For this reason; while the L* and b* decreased, a* increased of the bitter orange sour and bitter orange sauce supplemented with CMP. There were significant differences ($p < 0.05$) in terms of L*, a* and b* values of the bitter orange juice from the bitter orange sour and the bitter orange sauce supplemented with CMP. The darkest sample acquire on bitter orange sour (control) which was evaporated to 65 ^{0}Brix. Because the longer concentration time was caused to the darkening of the samples with non-enzymatic browning reactions (36). Since supplemented with CMP of bitter orange sauce, attribute consistency to the product naturally, it was evaporated for a shorter time (55 ^{0}Brix). Thus, it was exposed to less heat treatment and lower change in its lightness (L*) according to the control.

Sensory Evaluation

The analysis results of the hedonic sensorial characteristics of the samples were given in Table 4. When the samples were evaluated in general, it was observed that there was no significant difference statistically between control and bitter orange sauce supplemented with CMP on sensory properties ($p>0.05$), except the color. The color of bitter orange sauce supplemented with CMP was liked more than the control and also consistency, taste and overall acceptability. Only, the odour of control was liked more than the bitter orange sauce supplemented with CMP, but there was no significant difference statistically. So, CMP did not cause considerable any negative changes in odour.

According to the analysis results ranking test the samples from the most liked to the least liked were listed as pomegranate syrup, bitter orange sauce supplemented with CMP and control. It was thought that the reason why the pomegranate syrup sample was mostly chosen in the first place was that it contains additives such as sugar or glucose-fructose syrup, acidity regulator, colorant and thickener. But, in the bitter orange sauce with CMP, no synthetic additives were used and the desired consistency was obtained with the natural CMP which had low cost and was rich in nutrients.

Table 3. Instrumental color values of the samples

Samples	Hunter Color Values		
	L*	a*	b*
Bitter orange juice	23.56±0.33^{a}	1.56±0.05^{a}	34.85±2.96^{a}
Bitter orange sour (Control)	1.90±0.38^{b}	8.24±0.42^{b}	4.43±0.80^{b}
Bitter orange sauce with CMP	7.69±0.71^{c}	11.89±1.56^{b}	2.68±0.38^{b}

There is no difference between the averages in the same column and shown with the same letters $p<0.05$

Table 4. Sensory analysis results of the samples

Samples	Color	Consistency	Odour	Taste	Overall acceptability	
					Simple	With Salad
Control	3.40±1.18^{a}	3.47±1.36^{a}	3.67±0.90^{a}	3.07±1.28^{a}	3.27±1.03^{a}	3.67±1.29^{a}
Bitter orange sauce with CMP	4.27±0.80^{b}	4.00±1.07^{a}	3.47±0.74^{a}	3.80±1.26^{a}	3.80±1.20^{a}	4.00±1.20^{a}

There is no difference between the averages in the same column and shown with the same letters $p<0.05$

DISCUSSION AND CONCLUSION

In this study, CMP-added natural bitter orange sauce was produced with the traditional method. So far, this study is the first research in the production of bitter orange sauce, and natural carob molasses pulp was used as a thickener agent. The sauce produced gives a sour taste similar to pomegranate sauce and does not contain any synthetic additives that can be consumed primarily in green vegetable salads, kinds of pasta or other desired food items. According to the results of the analysis, these positive effects were observed with the addition of carob molasses pulp to the product. The product obtained a functional property with an increase in phenolic content and antioxidant activity. The consistency was increased naturally, in this way, the consistency of the sauce sample with CMP which was evaporated up to 55 ^{0}Brix became an equivalent control sample which was evaporated up to 65 ^{0}Brix. In terms of sensory properties (color, consistency, taste and overall acceptability, bitter orange sauce supplemented with CMP were appreciated more. On the other hand, other positive effects of the addition of carob molassespulp on the economy and environment: Contribute to the country's economy and industrial development by using carob molasses pulp which was generally used as animal feed or waste. It is possible to produce bitter orange sour supplemented with CMP using the traditional method, but using technology to produce under a vacuum will cause less damage to product quality characteristics.

Inference for Gastronomy

Bitter orange fruits are identified with the Mediterranean region and come into prominence, and the grown products are determinant in the cuisine of the region. Local products in Turkish cuisine can be used more effectively in the image of gastronomy by increasing their awareness. For this reason, the consumption of bitter orange sour should not only belong to the Mediterranean region; but should also be promoted in other regions and its use should be popularized. In this study, it was determined that carob molasses pulp (CMP) can be applied in the production of bitter orange sauce. A new sauce that is produced using cheap and natural CMP has been developed for today's eating habits. This product will be a guide for those working both in the field of gastronomy and in the research-development (R&D) departments of food. In addition, sours used in Turkish cuisine and the characteristics of sours have contributed to the knowledge in the literature.

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