

UTILIZING WHEY IN FORMULATING BEVERAGE ENRICHED WITH ANTIOXIDANTS (FUNCTIONAL BEVERAGE)

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ABSTRACT

Nutritional beverage will add value for consumer and provide food processors with enriched functionality and ease of use. A beverage was formulated using milk as a model for understanding how proteins, carbohydrates, fats, stabilizer, emulsifiers and salt work together in a fluid-based system. The study was aimed to use sweet whey- waste component from cheese manufacture- and enriched with other components; as a base component for this beverage. Whey was supplemented with ingredients to give healthy beverage. Different ingredients and concentrations were under test. The formula that gave a balance of nutrients, flavor and mouth feel; was concentrated whey (UF) 3% protein; included these ingredients; (0.5%) olive oil mixed with some essential oils (caraway oil, chamomile oil, ginger oil, fennel oil and peppermint oil); (0.5%) Nitrosoft 55 (gum Arabic and monoglycerides); 3.24% protein (3% whey protein and 0.24% soy protein), 6.7% carbohydrate (honey and lactose) as well as antioxidant vitamins (E and C), with carrot as a source of carotenoids and flavor. This formula gave a nice homogeneous flavor, enriched mouth feel without settling or phase separation. The components in this beverage have the potential to antioxidants.

Key words: Functional foods, Whey, Essential Oils, Vegetable Oils, Soy protein, Whey protein, Antioxidant potential

INTRODUCTION

Functional foods are only one aspect of a complete diet and healthy lifestyle. Under current regulations, functional foods or components can be placed into a number of existing regulatory categories, including conventional foods, food additives, dietary supplements, medical foods, or foods for special dietary use. These

categories are implemented under regulations from the Food and Drug Administration's (FDA). Development of functional food products will continue to grow throughout the 21st century as consumer demand for healthful products (Milner, 2000).

Current trends in dairy processing have justified the need for more functional ingredients. Every body-all cells in

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our bodies require antioxidants that protect cells and DNA from damage by oxidative stress (free radicals) and keep our immune systems functioning properly. As we age, our pools of antioxidants decrease. Supplementation with individual antioxidants may not be the best way to strengthen the antioxidant defense, so we need the balance of the mixture because there are certainly other components have the potential to be antioxidant.

Formulating this beverage requires a through technical knowledge of the ingredients, of their interactions with each other, and ways they're affected by the processing treatment used. Knowing when, and how, to incorporate an ingredient into a mix also is vital to preserve its functional characteristics. Whey that used as a base in the beverage formula contains lactose, proteins and minerals: Whey protein is a rich source of thiols. Colbert and Decker, 1991; have suggested that whey proteins donate hydrogen to reduce free radicals. Fat sources for beverage formula must be under test to choose the best one for this formula. These include essential oils, olive oil and black cumin oil. Essential oils are used as natural additives in many foods and play different roles. They are usually added in small amounts, so that the addition of the antioxidant fraction is much lower. Nevertheless, their effect should not be ignored, as they act in combination with various native antioxidants or synergists. Combination of natural essential oils with olive oil was used into beverage supplement formula. Olive oil is relatively stable as it is mainly monounsaturated and contains several natural antioxidants of the tyrosol series. *Nigella Sativa* oil that known in Egypt "Habbat al-baraka or black cumin seeds", has enormous suc-

cess in tumor therapy without the negative side effects of common chemotherapy. The active components in nigella seeds are the volatile oils thymoquinoline and dithymoquinoline. In same time, *Nigella Sativa* oil is rich in long chain fatty acids, which have anti-denaturation activity, protect against protein denaturation as mentioned by (Saso *et al* 1999). Soy and whey proteins provide rich sources of branched-chain amino acids. Sweetness is an important feature for almost all beverages. Honey could be used as sweeteners and antioxidant.

The presence of hydrocolloid stabilizers is important to stabilize beverage to sedimentation. Adding an emulsifier will prevent fat ringing and give a nice homogeneous product. Combination of emulsifier and stabilizer were used to act synergistically. We tested three types of these combinations. The aim of this study was, how making balance between nutrients, flavor, and mouthfeel through formulated beverage, have different natural sources of antioxidants as functional beverage. The growth of the functional beverage market has far outpaced the beverage category as a whole and has led all other functional food segments (Hollingsworth, 2000).

MATERIAL AND METHODS

Chemicals

Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA) from (Merck), hydrochloric acid (HCl), barium chloride dehydrate, ferric sulphate $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, ammonium thiocyanate. Chloroform, and methanol were of analytical grade. 1,1,3,3-tetraethoxypropane (Sigma) was used as a standard solution. Natural vita-

min E (d-alpha-tocopheryl acetate), vitamin C (L-ascorbic acid sodium salt) and oleoleic acid (99% purity) were purchased from Sigma Chem.Co. L-cysteine hydrochloride monohydrate, (from Aldrich) was used to make a standard cysteine-HCl curve. Tween 20 was from Merck. Genu Pectin type JM was from Københagens Pektinfabrik, Lille Skensved, Denmark. Gum Arabic with monoglyceride (Nitrosoft 55) was from Mifad Co, Egypt. Also, Lacta 501 (Gelatin and monoglyceride) was from Mifad Co. Egypt.

Ingredients

Sweet whey (cheese made from pasteurized milk-obtained from Arab Dairy factory) that used in preparing beverage was ultrafiltered at 40°C using DDS-Lab. 20 ultrafiltration unit, to get whey protein concentrate (3% protein). Essential oils include caraway oil, chamomile oil, ginger oil, fennel oil, peppermint oil and up to 250mg with edible oil' olive oil', from Arab Co. for Gelatin and Pharm, Products, Alex.-A.R.E. Olive oil purchased from supermarket. *Nigella Sativa* oil was gotten after cold pressing. Soy bean (48% protein, 6% fat) was brought from Nutrition Institute, El-Giza, Egypt. Honey was from Agriculture Research Center, Giza, Egypt. Fresh carrot was used, after drying in oven at 40°C under vacuum, as source for β -carotene and flavor.

Methods

Evaluating the antioxidant potential of oils

Essential oils do not dissolve in water, so that we diluted them with vegetable oil (olive oil). Sixty mg of each essential

oils, caraway oil, chamomile oil, ginger oil, fennel oil, peppermint oil were mixed and up to 500 mg with edible oil' olive oil'. Olive oil and *Nigella Sativa* oil, also were tested. Oleoleic acid emulsion was used to choose the best antioxidant potential oil.

Oleoleic acid emulsion preparation

Oleoleic acid emulsion containing 100 μ L of oleoleic acid mixed with 400 μ L ethanol that dispersed with 40 μ L Tween 20 and 460 μ L sodium phosphate buffer (50 mM, pH 7.4). Oleoleic acid emulsion system includes tested oil 0.5:1 (oil/ethanol, v/v).

Oxidative samples

The antioxidant activity samples were determined by exposing treatments to different lipid oxidation stress; three oxidative factors for 1-10 days, by raising the temperature, exposure to UV light and aeration as a source of oxygen. The first factor was carried out by incubating the emulsion in oven at 60 °C, in the dark and the second factor was, by exposure emulsions to fluorescent light, overnight and the last one was by injection air inside the emulsion (3 h).

Lipid Peroxidation Assay

Peroxide value determination (PV)

Hydrogen peroxide production was assayed by the spectrophotometric method (IDF method, 74A:1991), by using Shimadzu spectrophotometer Model 240.

Measuring thiobarbituric acid reactive substances (TBARS)

The most widely used method is the TBA reaction with malondialdehyde (MDA) the first breakdown product from the action of "free radicals". This method is of particular interest because of its procedural simplicity and nanomolar sensitivity. The lipid peroxidation products, was assayed by the thiobarbituric acid (TBA) test (Draper *et al* 1993).

Process and Formulating Beverage

Safe levels of intake must be considered in functional foods in the context of a healthy diet. The safety levels that permitted from Food and Drug Administration's (FDA) were taken in consideration with the bioactive components used to supplement the whey. Beverage was formulated and processed by the following ingredients; to make two main treatments, the first its base was whey (0.6% protein) and the second its base was concentrated whey (3.0 % protein). Three types of combination stabilizer and emulsifier were tested. Formula supplemented with 0.5% accepted oil was mixed with soy protein and vitamin E (200 mg) and blended together in small quantities of whey until the oil droplets broken up into smaller particles that cannot be seen by the naked eye. Honey (3 g) was dispersed into small quantity of heated whey (40°C) to dissolve, then ascorbic acid (0.1 g) was added and mixed. Hydrophobic and hydrophilic aqueous phases were mixed with adding the mixture of prepared stabilizer-emulsifier (5%), then made up to 100 ml whey or (whey concentrate) for each sample followed by mild heating to (40°C) and blended.

Three mixture of gum and emulsifier were tested; Lacta 501 (Gelatin and monoglyceride), Nitrosoft 55 (gum Arabic and monoglyceride), and (pectin JMJ and monoglyceride). Heat treatment was performed at 85°C for 15 seconds then cooled in ice and held at 5°C until sensory analysis.

Physical properties of the beverage

1. Sedimentation

The stability of beverage components was measured by determination of sedimentation, according to Towler, (1984). Beverage samples were tested at the first day (within 24 h of pasteurization) then after 1, and 2 weeks of cooled storage.

2. Sensory Evaluation

Organoleptic assessment of the beverage samples were carried out by 10 staff members at Dairy Dept. of National Research Centre, for appearance (40 points), colour (20 points), and flavor (40 points).

3. Beverage viscosity

Höppler viscometer was used in measuring the viscosity of acceptable beverage. Viscosity in centipoise (cp) was estimated at 20°C using ball with diameter 15.805 mm and Sp.gr. 2.406.

Chemical composition of the beverage

Total solids, protein contents of the beverage were determined according to AOAC methods (1990). Total carbohydrate was determined according to Taylor, (1995). Fat content and pH were also determined, according to Ling, (1963).

These analyses were carried out for accepted sample that obvious through physical assays.

RESULTS AND DISCUSSION

Lipid oxidation in lenoleic acid emulsion including different oils

Lenoleic acid emulsion was oxidized by light, temperature (incubated in oven at 60°C, in the dark) and oxygen, generating hydroperoxides that decompose through secondary reaction to aldehydes, ketones, acids and alcohols (Shahidi & Wanasundara, 1996). The estimation was run at 0.0, 3, 5, 7 and 10 days. The obtained results are shown in Fig. (1). Oxidation of lenoleic emulsion included olive oil gave the lowest level for peroxide concentration, with three catalytic factors, whereas emulsion included other oils gave more peroxide concentrations. The ability for decreasing peroxide concentration for olive oil started after 3 days from oxidation. Although the PV measurement is a useful method of monitoring oxidative deterioration of oils, it should normally be combined with a method of monitoring secondary oxidative products to provide a fuller picture of the progress of oxidation as shown in Fig. (2). Determination of TBARS value was used to monitor the extent of oxidation. Results indicate that olive oil mixed with essential oils was rich in antioxidant, which had been shown in reducing or retarding the lenoleic acid emulsion system oxidation with all oxidative stress. Essential oils were act in combination with various native antioxidants or synergists.

Sedimentation

Stability is important for maintaining appearance, since beverage water phases contain suspended fiber, flavor and protein. Results show that combination of gum Arabic with monoglyceride (Nitrosoft 55) gave beverage without settling or phase separation. Gum Arabic interacts with water, allowing them to act as stabilizers in aqueous food systems by preventing settling, phase separation. The presence of hydrocolloid stabilizers is important to stabilize beverage to sedimentation. Adding an emulsifier also prevented fat ringing and gave a nice homogeneous product. Using gelatin reduced settling of carrot particles, whereas using pectin gave phase separation.

Sensory Evaluation

The most important sensations contributing to beverage flavor are taste, smell and feel. The organoleptic evaluation of the beverage was carried out when fresh and after cold storage, 1-wk and 2-wk. The organoleptic properties of beverages made with Lacta 501 and Nitrosoft 55 presented in (Table, 1) indicate that beverage processed from concentrated whey (3% protein) using Nitrosoft 55 (gum Arabic and monoglyceride) was the best beverage. The presence of antioxidants did not affect the descriptive sensory of the beverage. Carrot showed an influence on flavor and color and that was acceptable. Although *Nigela Sativa* oil worked well as antioxidant, the panelists refused the taste of beverage containing it.

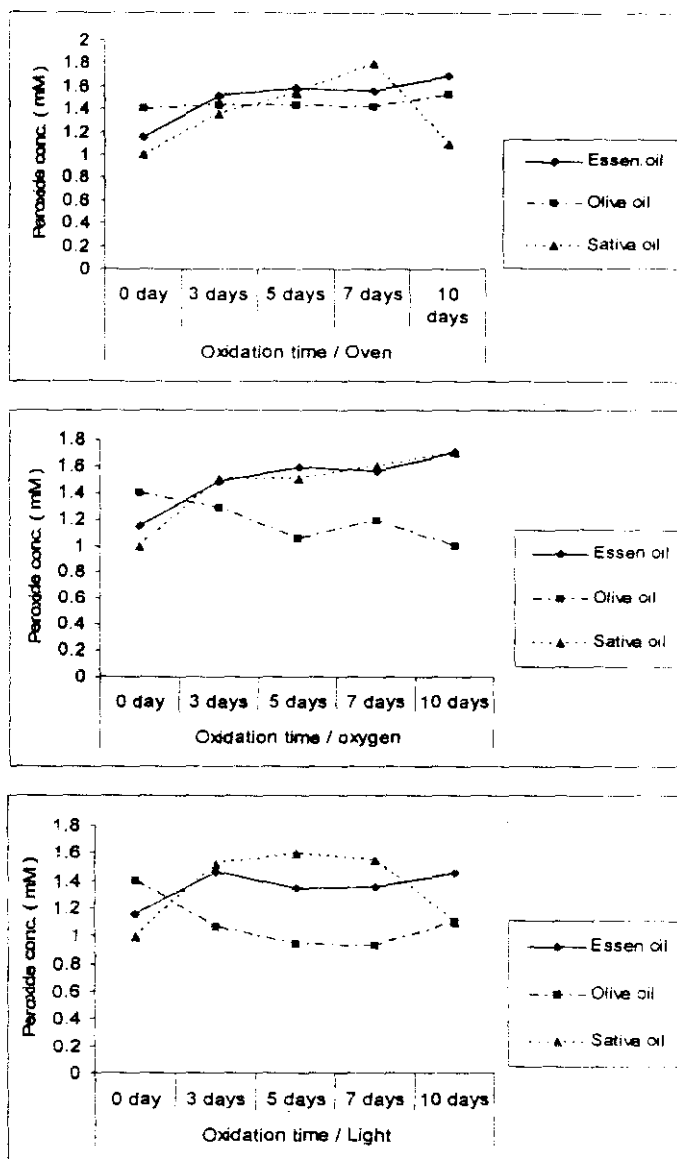


Fig. 1. Peroxide concentration in lenoleic acid emulsion produced as a result of oxidizing by elevated temperature, aeration and exposure to light as pro-oxidants

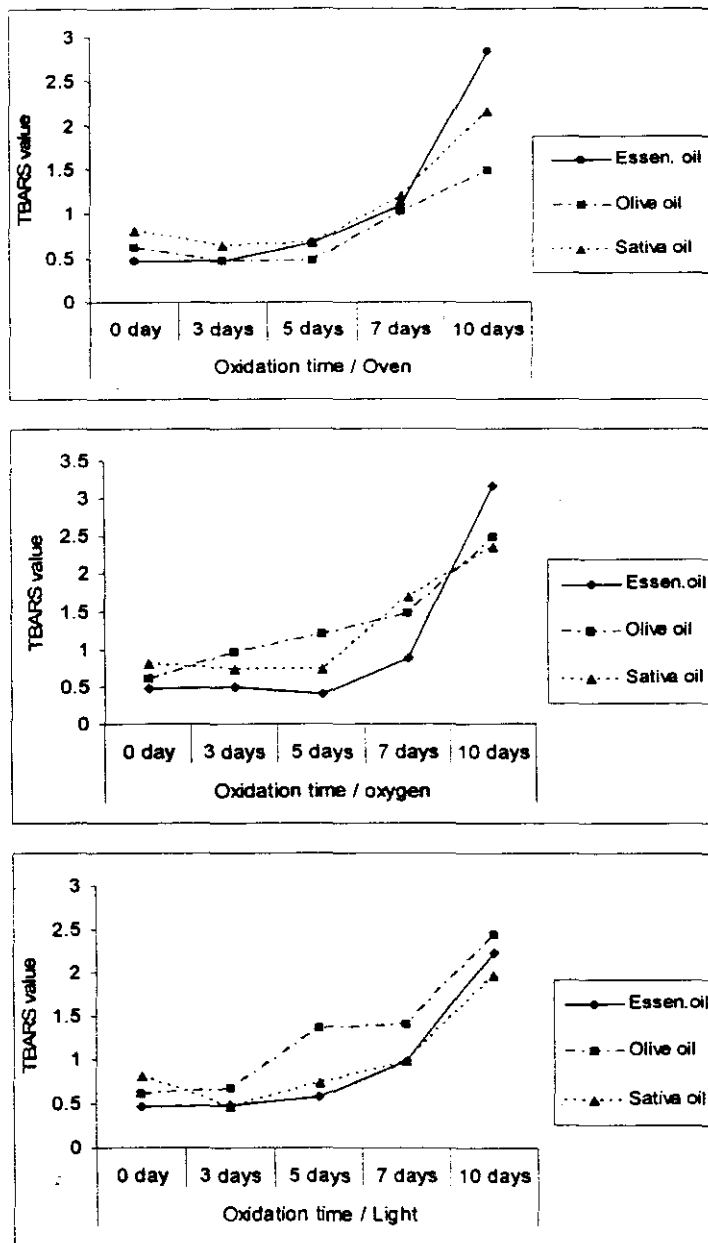


Fig. 2. Thiobarbituric acid reactive substances (TBARS value) in lenoleic acid emulsion produced as a result of oxidizing by elevated temperature, aeration and exposure to light as pro-oxidants

Table 1. Organoleptic properties of formulated beverage, at different storage periods

| Storage period (days) | Appearance (40) | | Colour (20) | | Flavour (40) | | Total (100) | |
|--|-----------------|--------------|-------------|--------------|--------------|--------------|-------------|--------------|
| | Lacta 501 | Nitrosoft 55 | Lacta 501 | Nitrosoft 55 | Lacta 501 | Nitrosoft 55 | Lacta 501 | Nitrosoft 55 |
| Beverage using whey (0.6 % protein) | | | | | | | | |
| 0 | 33 | 35 | 19 | 19 | 34 | 35 | 89 | 86 |
| 7 | 32 | 34 | 18 | 18 | 32 | 35 | 87 | 82 |
| 15 | 30 | 34 | 15 | 17 | 31 | 33 | 84 | 76 |
| Beverage using concentrate whey (3.0 % protein) | | | | | | | | |
| 0 | 32 | 37 | 20 | 20 | 35 | 27 | 94 | 87 |
| 7 | 30 | 36 | 18 | 18 | 35 | 36 | 90 | 83 |
| 15 | 30 | 35 | 16 | 17 | 34 | 35 | 87 | 80 |

Appearance = homogeneity, precipitate and clearness

Flavour = taste, odor and feeling

Beverage viscosity

Viscosity is a primary factor in the prevention of settling and the aggregation of solids suspended in drinks. The viscosity of the beverage is exponentially related to the concentration, chemical and biochemical changes in the beverage, besides heat treatment. However, suspending solids and hydrocolloids can modify a drink by adding viscosity. The viscosity of beverage made from whey mixed with Lacta 501 was 1.66 cp, while that mixed with Nitrosoft 55 was 1.98 cp. The viscosity of beverage made from whey concentrate mixed with Lacta 501 was 3.5 cp and for that mixed with Nitrosoft 55 was 4 cp. Generally, the best physical properties was related to beverage containing essential oil mixed with olive oil, concentrated whey 3% protein and mixture of gum Arabic with monoglycerides.

Chemical composition

Chemical composition of this beverage formula has TS 12.57%, fat 1.03 %, carbohydrate 6.5%, total protein 3.24%, vitamin E approx. 200 mg, vitamin C approx. 100 mg. and the pH was 5.2. The product was characterized as clean, high quality and delicately balanced between acidity, sweetness and flavor.

The nutritional value of the formulated beverage

The bioactive components used to supplement the whey taking in consideration the safety levels that permitted from Food and Drug Administration's (FDA). These ingredients include lactose that provides calories in an easily available form with slower digestion and absorption (compared to glucose), benefits intestinal calcium, magnesium and zinc

absorption. Whey protein is a rich source for free sulfhydryl groups from cysteine that are effective at inhibiting lipid autoxidation (Taylor and Richardson, 1980). Whey protein is the only protein type that cannot be obtained from regular food in significant amounts. Whey protein appears to be unique among proteins in its ability to improve immune function, elevate cellular glutathione levels (Counous, 2000) an antioxidant enzyme containing cysteine, and extend lifespan. Olive oil has flavenoid; polyphenols that consider natural antioxidants (Kellie *et al* 2001). Essential oils used into beverage supplement formula as a flavoring besides its acting in combination with various native antioxidant or synergists. Soy protein is a high quality protein has efficiency in cholesterol-lowering, the most well-documented physiological effect (Albertazzi *et al* 1998 and Meister, 2002). Honey contains various water-soluble antioxidants compounds, flavanoids and phenolic compounds. Vitamins A, E and C are reported to act as effective antioxidant of major importance for protection against diseases and degenerative processes caused by oxidative stress (Olas & Wachowiej, 2002 and Chaudiere & Ferrari-Illiou, 1999).

CONCLUSION

Beverage needs balance of nutrients, flavor, texture and mouthfeel often serves as the "gold standard" for many of today's formulated drinks. Fortification with antioxidants vitamins was necessary to act as free-radical scavengers. Low level of soy protein was used to achieve the level required in the health claim, but another ingredient was needed to hide some soy-bean flavor. Honey and carrot were used.

This beverage can promote healthy complexion, decrease in blood pressure, strengthened immunity.

Adding antioxidants during processing can still play a very important role since the added compounds have the potential for enhancing the activity of the inherent antioxidants systems. More work is needed to define the optimum dietary combinations and / or the minimum levels of the compounds in the dairy products necessary for obtaining the greatest stability in the resulting product. This may involve defining interactions of dairy products components on the uptake on the desired compounds.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م. ٥٠ ، ع (١) ، ١٩٧ - ٢٠٧ ، ٢٠٠٥

استخدام الشرش فى تركيب مشروب غنى بمضادات الأكسدة (مشرب وظيفى)

[١٥]

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المشروب بفيتامينات (ج ، هـ) واستخدم الجزر المجفف كمصدر للكروتين وللنكهه (١%) . تم تجربة ثلاث مخاليط من المثبت مع المستحلب (جيلاتين مع الجلسريدات الأحادية) ، (البكتين مع الجلسريدات الأحادية) ، (الصمغ العربى مع الجلسريدات الأحادية) بنسبة ٠,٥%. وأجريت عملية تصنيعه. تم اختبار الخواص الطبيعية للمنتج. وكانت أفضل تركيبة أعطت الاتزان ما بين المكونات المضافة ونالت الاستحسان، المنتج المستخدم فيه الشرش (٣% بروتين) ومخلوط (الصمغ العربى مع الجلسريدات الأحادية) والتي أعطت التركيب الأتى :

جوامد كلية ١٢,٥٧% ، دهن ١,٠٣% ، كبروهيدرات ٦,٥% ، بروتين كلى ٣,٢٤% فيتامين ٥ ٢٠٠ ملجم/١٠٠ مل ، فيتامين ج ١٠٠ ملجم /١٠٠ مل ودرجة ال pH ٥,٢ .
يمثل هذا المشروب مصدر جيد للبروتينات المثبته لأكسدة الليبيدات فى الخلية ، والمخفضة لنسبة الكوليسترول فى الدم كما يحتوى على المركبات الفينولية والتي لها وظيفة تضاد للأكسدة .

تم استخدام الشرش الحلو كمكون أساس فى تركيب مشروب غنى بمضادات الأكسدة بالاستعانة بشكل تركيب اللين كموديل سائل مركب مكون من دهن- بر وتين- كبروهيدرات- فيتامينات حيث تم تدعيم الشرش بمصادر مختلفة غنية بمضادات الأكسدة . استخدم فى التجربة الشرش (٦% بروتين) ، والشرش المركز (٣% بروتين) كأساس فى المكون وتم تدعيمها بالمصادر الطبيعية الغنية بمضادات الأكسدة. تم تجربة ثلاث مصادر للدهن (مخلوط من زيوت طيارة : زيت الكراوية، زيت الكاموميل، زيت الزنجبيل ، زيت الشمر ، زيت النعناع ٦٠ ملجم من كل منهم واستكملوا بزيت الزيتون الى ٥٠٠ ملجم) ، زيت حبة البركة ٥٠٠ ملجم ، زيت الزيتون ٥٠٠ ملجم لكل ١٠٠ مل مشروب. وتم اختبار كفاءة كل منهم كمضاد للأكسدة وكان أفضلهم زيت الزيتون المخلوط بالزيوت الطيارة. استخدم بروتين الصويا (٠,٥ جم) كمصدر للبروتين يعمل مع بروتين الشرش كما استخدم العسل الأبيض (٣%) كمصدر للتخلية ونشاطه كمضاد للأكسدة . تم تدعيم

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