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(54) Title: COMPOSITION OF TASTE OR FLAVOUR ENHANCER, PREPARATION AND USE THEREOF

(57) Abstract: The invention relates to the novel specific composition of umami and kokumi taste or flavour enhancer comprising: (i) one or more yeast extracts and/or hydrolyzed vegetable protein as a source of natural glutamic acid; (ii) one or more yeast extracts as a source of natural 5'-ribonucleotides; (iii) glutathione; or yeast extract as a source of natural glutathione; (iv) nutritionally acceptable calcium or magnesium salt, or their combination with weight ratio within 10:1 to 1:5; and optionally, sodium chloride and one or more auxiliary food ingredient that provides final form of the composition suitable for practical use as cubes, powder, granules, paste, suspension, emulsion, or solution. The composition of the present invention acts as efficient umami and kokumi taste enhancer suitable for use as either table-top seasoning or as flavour enhancer in various food products.

# COMPOSITION OF TASTE OR FLAVOUR ENHANCER, PREPARATION AND USE THEREOF

## DESCRIPTION

### Technical Field

The invention relates to a new composition of taste or flavour enhancer based on particular types of yeast extracts (YE; YEG, YER) based on natural glutamic acid (GA), natural ribonucleotides (RN), and glutathione (GSH) or glutathione-based yeast extracts (YEGSH), and nutritionally acceptable calcium and/or magnesium salts, its preparation and use.

### Technical Problem

Technical problem is related to more efficient umami and kokumi taste enhancement without the use of stigmatized well-known flavour enhancer monosodium glutamate (MSG) and/or taste enhancing synthetic aroma chemicals.

Furthermore, "more efficient" taste enhancement, as used hereby, means to achieve better umami and kokumi taste enhancement than those obtained with the use of known combinations of naturally occurring glutamate (GA), 5'-ribonucleotides (RN), and glutathione (GSH) sources, such as various yeast extracts (YE; YEG, YER, YEGSH).

The solution of the present invention is based on the use of a novel specific composition of umami and kokumi taste or flavour enhancer suitable for practical use in the form of: cubes, powder, granules, paste, suspension, emulsion, or solution; where said composition has ingredients (i) and (ii):

(i) one or more yeast extracts (YEG), or hydrolysed vegetable protein, or combination thereof as a source of glutamic acid (GA);

(ii) one or more yeast extracts (YER) as a source of 5'-ribonucleotides (RN); and

where said composition further comprising ingredients (iii) and (iv):

(iii) glutathione (GSH), or yeast extract as a source of glutathione (YEGSH);

(iv) nutritionally acceptable salt selected from: calcium salts, magnesium salts, or their combination with weight (w/w) ratio calcium : magnesium salts in the range 10:1 to 1:5; and

optionally, sodium chloride, one or more auxiliary food ingredients that provide final form.

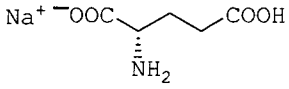
The composition of the invention provides effective umami and kokumi taste enhancement in various savoury applications.

**Previous State of the Art**

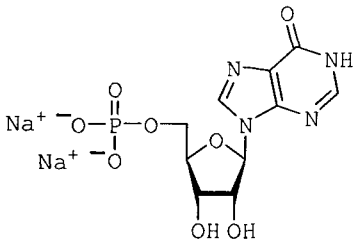
Taste or flavour enhancers represent an important group of food products that are used either as sole seasoning agent or in various compositions of food products. They provide taste enhancement to food products in which they are applied. Probably the largest importance belongs to the umami taste enhancers that are employed in various savoury applications. The most known umami flavour enhancer is monosodium glutamate (MSG) which is stigmatized in recent years due to its negative health effects, when consumed in large quantities; see literature reference 1:

1) R. S. Geha, A. Beiser, C. Ren, R. Patterson, P. A. Greenberger, L. C. Grammer, A. M. Ditto, K. E. Harris, M. A. Shaughnessy, P. R. Yarnold, J. Corren, A. Saxon: Multicenter, double-blind, placebo-controlled, multiple-challenge evaluation of reported reactions to monosodium glutamate, *J. Allergy Clin. Immunol.* **106** (2000) 973-980.

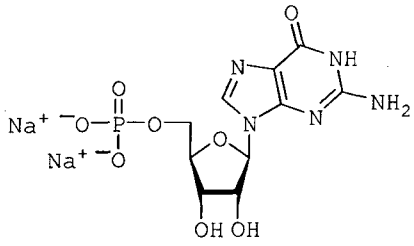
Also, 5'-ribonucleotides (RN) such as disodium inosinate (DSI) and guanylate (DSG) act as co-enhancers to MSG, providing additional synergistic action on glutamate umami taste enhancement perception; see literature reference 2:



MSG



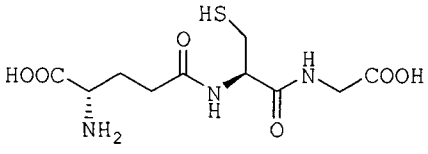
DSI



DSG

2) S. Yamaguchi, K. Ninomiya: Umami and Food Palatability, *J. Nutr.* **130** (2000) 921S-926S.

Further development of taste enhancers was lead to kokumi substances and compositions which additionally improve umami taste, mouthfulness, and overall deliciousness in various savoury applications. Thus Maruyama and co-workers described different kokumi substances including naturally occurring tripeptide γ-L-glutamyl-L-cysteinyl-glycine (Glu-Cys-Gly), known under trivial name glutathione (GSH); see reference 3:



GSH

3) Y. Maruyama, R. Yasuda, M. Kuroda, Y. Eto: Kokumi Substances, Enhancers of Basic Tastes, Induce Responses in Calcium-Sensing Receptor Expressing Taste Cells, *PLOS One* **7** (2012) e34489.

According to the reference 3, GSH does enhance umami taste. Therefore, it is known to those skilled in the art that the combination of known umami taste enhancer composition of glutamate + 5'-ribonucleotides, when combined with glutathione (GSH), provides further umami taste enhancement with additional kokumi effect.

Regarding the mechanism of glutathione (GSH) kokumi action, it was speculated that this is realized upon its stimulating or agonistic

action on calcium-sensing receptors (CaSR); see literature references 3 and 4:

4) M. Kuroda, N. Miyamura: Mechanism of the perception of "*kokumi*" substances and the sensory characteristics of the "*kokumi*" peptide γ-Glu-Val-Gly, *Flavour* **4** (2015) 11; doi: 10.1186/2044-7248-4-11.

However, due to a very complex role of calcium-sensing receptors in different tissues, despite their influence in kokumi taste perception, no definite conclusion can be outlined from existing literature data on their (CaSR) role on umami and kokumi taste perception.

Beside monosodium glutamate (MSG), 5'-ribonucletides (RN), and their nutritionally acceptable salts, various yeast extracts (YE) are commonly employed as, so-called, "clean label" or "natural flavour" alternatives to the former food additives which are perceived by consumers as "synthetic" or "unnatural". There exist various compositions of seasoning agents and taste or flavour enhancers based on yeast extracts (YE).

Yeast extracts (YE) are manufactured from autolyzed suspension of common or bakery yeast (*Saccharomyces cerevisiae*), or torula yeast cells by filtration to remove water insoluble components, e.g. cell walls, and subsequent concentration or spray draying of resulting filtrate. Thus obtained highly viscous liquid or powderous products are widely employed in the food industry as said "clean label" umami taste enhancers. They contain relatively high percentage of naturally occurring glutamic acid (GA) and/or other umami amino acids like aspartic acid, as well as 5'-ribonucleotides (RN), and/or glutathione (GSH), or similar sulphur-containing small molecules.

Various hydrolyzed proteins are also used as sources of naturally occurring umami taste enhancer glutamic acid. The most important are different hydrolyzed vegetable proteins (HVP), mainly produced from maize, wheat, or pea proteins.

Kimizuka and co-workers in EP0181421A1 disclosed flavour enhancing seasoning composition which comprises:

(i) glutamic acid (GA), its salts, or

(ii) 5'-ribonucleotides (RN): inosinate (IMP) or guanylate (GMP), and

(iii) glutathione (GSH), oxidized glutathione (GSSG), glutathione salts or other compounds as taste enhancer; see literature reference 5:

5) EP0181421A1, A. Kimizuka, M. Sakaguchi, R. Miyajima, Y. Ueda, N. Mori: Flavor enhancing seasoning and foods containing them; Ajinomoto Co. Inc. (JP).

In a similar manner, Kawase and coworkers described the process and the composition for dehydrated instant soup or sauce containing taste enhancer of the following formulation:

(i) monosodium glutamate (MSG) as glutamate source;

(ii) ribonucleotides (RN) like DSI or DSG; and

(iii) sulphur compounds including glutathione (GSH); see literature reference 6:

6) EP1075798A1, H. Kawase, M. Hasegawa, H. Sasaki, N. Miyamura: Process for production dry instant soups and sauces, Ajinomoto Co. Inc. (JP).

According to our best knowledge the latter two documents represent the closest prior art to the present invention.

Furthermore, Haibin and Jing in CN1387794A disclosed a composition of health table salt comprising sodium chloride (NaCl), potassium chloride (KCl), amino acids, peptides, nucleic acid seasonings, calcium salt (e.g. calcium citrate), magnesium salt (e.g. $MgSO_4$), polysaccharide chitin, B-vitamins, sodium selenite ($Na_2SeO_3$), and potassium iodide (KI) or iodate ($KIO_3$). They stated that in this formulation, among amino acids, glutamic acid can be used, as well as glutathione as peptide.

However, this invention neither use natural sources of glutamic acid and ribonucleotides such as yeast extracts, nor recognize any synergistic action of calcium and/or magnesium salt on glutathione enhancement of umami and kokumi tastes perception of basic yeast extracts containing natural glutamic acid + 5'-ribonucleotides.

In addition, the state of art remains silent regarding the synergistic action of calcium and/or magnesium salt activity upon glutathione (GSH) kokumi action and its umami enhancing activity in glutamate + 5'-ribonucleotide(s) taste enhancing compositions that is the core of the present invention.

**Summary of Invention**

The present invention discloses the new specific composition of umami and kokumi taste or flavour enhancer suitable for practical use in the form of: cubes, powder, granules, paste, suspension, emulsion, or solution. Said composition has ingredients (i) and (ii):

(i) one or more yeast extracts (YEG), or hydrolysed vegetable protein (HVP), or combination thereof as a source of glutamic acid (GA);

(ii) one or more yeast extracts (YER) as a source of 5'-ribonucleotides (RN);

where said composition further comprising ingredients (iii) and (iv):

(iii) glutathione (GSH), or yeast extract as a source of glutathione (YEGSH);

(iv) nutritionally acceptable salt selected from: calcium salts, magnesium salts, or their combination with weight (w/w) ratio calcium : magnesium salts within the range 10:1 to 1:5; and

optionally, sodium chloride, one or more auxiliary food ingredients that provide final form.

Yeast extracts (YEG) used as a source of natural glutamic acid (GA) contain minimally 2% w/w of L-glutamic acid (GA). As alternative ingredient for yeast extract as a source of natural glutamic acid (YEG), any hydrolyzed vegetable protein (HVP) can be used.

Yeast extracts (YER) as a source of 5'-ribonucleotides (RN) contain minimally 1% w/w of natural 5'-ribonucleotides (RN) selected from the group consisting of: inosine 5'-monophosphate (IMP), guanosine 5'-monophosphate (GMP), or mixtures of these substances.

Yeast extracts (YEGSH) as sources of natural glutathione (GSH) contain minimally 0.5% w/w of glutathione.

Nutritionally acceptable calcium and/or magnesium salt involves all calcium or magnesium salts of nutritionally acceptable and essentially non-toxic acids.

The composition of the present invention optionally contains sodium chloride or other auxiliary food ingredients providing final form suitable for practical use as the taste enhancer: cubes, powder, granules, paste, suspension, emulsion, or solution.

The composition of the present invention acts as efficient umami and kokumi taste enhancer suitable for use in savoury applications.

**Detailed Description**

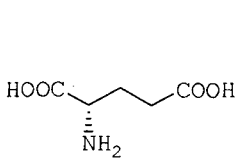
The present invention discloses the new specific composition of umami and kokumi taste or flavour enhancer suitable for practical use in the form of: cubes, powder, granules, paste, suspension, emulsion, or solution. Said composition has ingredients (i) and (ii):

(i) one or more yeast extracts (YEG), or hydrolysed vegetable protein (HVP), or combination thereof as a source of glutamic acid (GA);

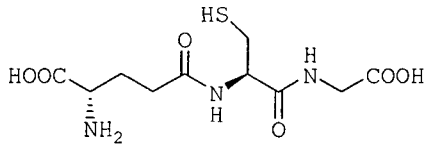
(ii) one or more yeast extracts (YER) as a source of 5'-ribonucleotides (RN);

where said composition further comprising ingredients (iii) and (iv):

(iii) glutathione (GSH), or yeast extract as a source of glutathione (YEGSH);




GA




GSH

(iv) nutritionally acceptable salt selected from: calcium salts, magnesium salts, or their combination with the weight ratio calcium : magnesium salts within the range 10:1 to 1:5; and

optionally, sodium chloride, one or more auxiliary food ingredients that provide final form.

In further embodiments, the present invention discloses the new specific composition of umami and kokumi taste or flavour enhancer containing:

(i) one or more yeast extracts (YEG), or hydrolysed vegetable protein (HVP), or combination thereof as a source of glutamic acid (GA); 5-75% w/w, preferably 25-50% w/w;

(ii) one or more yeast extracts (YER) as a source of 5'-ribonucleotides (RN); 5-75% w/w, preferably 10-25% w/w;

where said composition further comprising ingredients (iii) and (iv):

(iii) glutathione (GSH); 0.01-10% w/w, preferably 0.1-3% w/w; or yeast extract as a source of glutathione (YEGSH); 0.1-50% w/w, preferably 1-25% w/w;

(iv) nutritionally acceptable salt selected from: calcium salts, magnesium salts, or their combination with weight (w/w) ratio calcium : magnesium salts in the range 10:1 to 1:5; 1-50% w/w, preferably 5-25% w/w; and

optionally, sodium chloride, one or more auxiliary food ingredients that provide final form.

Yeast extracts (YE) that are employed in the present invention are derived from:

(1) the common yeast [*Saccharomyces cerevisiae*]; or

(2) torula yeast [*Candida utilis*].

Instead of yeast extracts, various deactivated yeasts (DY) can be used. Such products contain also water-insoluble parts of original yeast cells. For instance, DY of type Springalys D100/0-PW, the product of Bio Springer (France), standardized on 0% NaCl, 9.5% total nitrogen (N), and 60% protein, can be employed as alternative ingredient providing natural glutamic acid (GA), see Example 1.

As another alternative ingredient for yeast extract (YEG) based on glutamic acid (GA), various hydrolyzed vegetable proteins (HVPs) can be used. The latter can be derived from:

(i) cereals: wheat [*Triticum spp* L.], maize [*Zea mays* L.], barley [*Hordeum vulgare* L.], rice [*Oryza sativa* L.], oats [*Avena sativa* L.], rye [*Secale cereal* L.], millet, triticale [x *Triticosecale*], buckwheat [*Fagopyrum esculentum* L.], fonio [*Digitaria spp.* L.], quinoa [*Chenopodium quinoa* L.],;

(ii) beans: common bean [*Phaseolus vulgaris* L.], pea [*Pisum sativum* L.], soybean [*Glycine max* L.], peanut [*Arachis hypogaea* L.], or other edible beans with high protein content; and

(iii) nuts: almonds [*Prunus dulcis* Mill.], walnuts [*Juglans regia* L.], pistachio [*Pistacia vera* L.], as well as other edible nuts with high protein content.

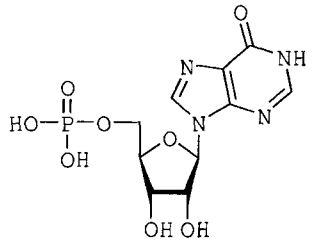
Depending on desired final physical form of the composition from this invention, yeast extracts (YE), deactivated yeasts (DY), and hydrolyzed vegetable proteins (HVPs) can be in commercially available physical forms of either: viscous solution, paste, or powder.

The yeast extract (YEG) as a source of glutamic acid (GA), deactivated yeasts (DY), or hydrolyzed vegetable protein (HVP) contains minimally 2% w/w of natural L-glutamic acid (GA).

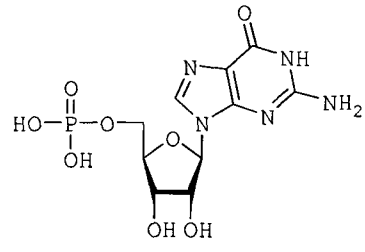
Under the term "glutamic acid", we understand free L-glutamic acid and/or glutamate anions in combination with any type of cation that naturally occurs in food ingredients such as yeast extracts (YE) and

hydrolyzed vegetable proteins (HVP), e.g. various protonized amino-group of amino acids, peptides, proteins, etc.

Yeast extracts (YER) as a source of 5'-ribonucleotides (RN) contain minimally 1% w/w natural 5'-ribonucleotides selected from the group consisting of: inosine 5'-monophosphate (IMP) or inosinic acid; guanosine 5'-monophosphate (GMP) or guanylic acid; or their mixtures.



**IMP**



**GMP**

It is clear to those skilled in the art that both IMP and GMP are present in the corresponding yeast extracts (YER) in the form of salts with various naturally occurring bases of the same extract, e.g. as sodium ($Na^+$), calcium ($Ca^{2+}$), lysine (Lys), arginine (Arg), histidine (His), various basic peptides, etc.

Yeast extracts (YEGSH) as a source of γ-L-glutamyl-L-cysteinyl-glycine (GSH), known under generic name glutathione (GSH), contain minimally 0.5% w/w of natural GSH.

All three substantially different types of yeast extracts (YE):

(i) YEG: a source of natural glutamic acid (GA);
(ii) YER: a source of natural 5'-ribonucleotides (RN), IMP and/or GMP; and
(iii) YEGSH: a source of natural glutathione (GSH); are widely available from several manufacturers under different brand names.

In the same time, many commercially available hydrolyzed vegetable proteins (HVP) derived from cereals, beans, and nuts, can be employed

instead of YEG, as alternative sources of naturally occurring glutamic acid (GA).

We have found that many of them can be successfully used for preparation of the present invention composition, as given in the Table 1; see Example 1.

**Table 1.** List of various commercially available yeast extracts, that can be used, among others, in preparation of the composition of this invention as YEG, YER, or YEGSH.

| No. | Type of yeast extract (or HVP) | Brand named commercial product |
|---|---|---|
| 1 | YEG [1] | [4] Ohly M1T, Ohly STT<br>[5] GISTEX LS Powder<br>[6] Springer 4115/0-MG-L, Pronal 5001/0-PW-L,Springer 2012/20-MG-L<br>[7] Leiber Bouillon G<br>[8] HVP Vitana<br>[9] Nutralys W |
| 2 | YER [2] | [10] Aromild<br>[11] Umamex 100LS<br>[7] Leiber Bouillon N, Leiber Bouillon HR, Leiber Aromor GS20<br>[12] High Lyfe 560A<br>[6] Springer 2006/0-MG-L<br>[5] MAXAROME Select Powder |
| 3 | YEGSH [3] | [12] High Lyfe 608A, High Lyfe 610A<br>[10] Aromild U-15<br>[6] Springer 4000 |

[1] Yeast extract (YE) or hydrolyzed vegetable protein (HVP) based on natural glutamic acid;

[2] Yeast extract based on natural 5'-ribonucleotides GMP and IMP;

[3] Yeast extract based on natural glutathione (GSH);

[4] Product of Ohly GmbH, Germany;

[5] Product of DSM Nutritional Products Ltd, Switzerland;

[6] Product of Bio Springer, France;

[7] Product of Leiber GmbH, Germany;

[8] Product of Rieber & Søn ASA, Norway/Czech Republic;

[9] Product of Roquette, France;

[10] Product of Kohjin LifeScience Co. Ltd., Japan;

[11] Product of Kerry Group plc, Ireland;

[12] Products of Lallemand Inc., Canada.

Sensory evaluation of various yeast extracts (YE) and some hydrolyzed vegetable proteins (HVP) is described in Example 1.

Nutritionally acceptable calcium and/or magnesium salt used in the composition of the present invention involves calcium or magnesium salts of nutritionally acceptable and essentially non-toxic acids.

Nutritionally acceptable calcium and/or magnesium salt is one or more ingredients selected from the group consisting of: calcium chloride ($CaCl_2$); magnesium chloride ($MgCl_2$); magnesium sulfate ($MgSO_4$); calcium lactate [$Ca(CH_3CH(OH)COO)_2$]; magnesium lactate [$Mg(CH_3CH(OH)COO)_2$]; calcium gluconate [$Ca(C_6H_{11}O_7)_2$]; magnesium gluconate [$Mg(C_6H_{11}O_7)_2$]; calcium citrates: calcium citrate [$Ca_3(C_6H_5O_7)_2$], calcium hydrogencitrate [$Ca(C_6H_6O_7)$], calcium dihydrogencitrate [$Ca(C_6H_7O_7)_2$]; magnesium citrates: magnesium citrate [$Mg_3(C_6H_5O_7)_2$], magnesium hydrogencitrate [$Mg(C_6H_6O_7)$], magnesium dihydrogencitrate [$Mg(C_6H_7O_7)_2$]; calcium aspartates: calcium aspartate [$Ca(C_4H_5NO_4)$], calcium hydrogenaspartate [$Ca(C_4H_6NO_4)_2$]; magnesium aspartates: magnesium aspartate [$Mg(C_4H_5NO_4)$], magnesium hydrogenaspartate [$Mg(C_4H_6NO_4)_2$]; calcium and/or magnesium containing mineral water salts; calcium-containing milk permeate; calcium caseinate; magnesium caseinate; calcium and/or magnesium-containing, essentially bromide-free, Dead Sea salt; various hydrates of above-mentioned salts such as calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), calcium lactate pentahydrate [$Ca(CH_3CH(OH)COO)_2 \cdot 5H_2O$], calcium gluconate monohydrate [$Ca(C_6H_{11}O_7)_2 \cdot H_2O$], magnesium lactate dihydrate [$Mg(CH_3CH(OH)COO)_2 \cdot 2H_2O$], etc.; or mixtures of these substances.

Various mineral waters are based on calcium and/or magnesium salts. For instance, many of them contains soluble calcium hydrogencarbonate [$Ca(HCO_3)_2$] and/or magnesium hydrogencarbonate [$Mg(HCO_3)_2$], together with smaller amounts of other salts like chlorides, sulfates, etc. Such mineral water salts can be used in preparation of the composition as a source of calcium and/or magnesium either:

(i) in a powder form to yield dry forms; or alternatively,

(ii) as mineral waters, with original natural concentrations of solubilized Ca-/Mg-salts, or in concentrated versions, serving in the same time as a diluents for preparation of liquid forms: solution or suspension.

Calcium-containing milk permeate or whey is a liquid or powderous product with predominant content of lactose (80%), small amounts of whey protein (3%), and up to 10% of milk minerals with calcium salts as important constituents. This milk permeate in either liquid or powderous form can be used as calcium-providing ingredient in the composition of the present invention for preparation of either liquid (solution or suspension) or solid final forms.

Calcium- and magnesium caseinates are products of milk processing, wherein the milk protein casein is converted to calcium or magnesium salt. These products can also be employed as calcium and or magnesium salts in the formulation of this invention.

Additionally, Dead Sea salt is an example of unusual sea salt with low content of sodium chloride but with predominant percentages of magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$). Since Dead Sea salt does contain significant content of bromides, raw product is essentially non-edible due to bromide toxicity. By removing bromides, resulting product, Dead Sea salt, essentially bromide-free, can be used in food products as a source of nutritionally important minerals: magnesium ($Mg^{2+}$), calcium ($Ca^{2+}$), chloride ($Cl^-$), and sodium ($Na^+$). Such bromide-free Dead Sea salt can be successfully employed as the calcium and magnesium source in the composition of the present invention.

The composition of the present invention can be used in the final forms of cubes, powder, granules, paste, suspension, emulsion or solution.

The final form of cubes comprises all generic 3D forms that are produced by moulding of thick warm mass of the composition. From practical point of view, one such cube is adequate dosage for one pot

of meal, usually adjusted for four persons. Beside cubes, such application form can be in the shape of balls, irregular cubes, parallelepiped, medallion-like pieces, etc. The corresponding shape and dimensions of such "cubes" are irrelevant and all remains within the scope of this invention.

The simplest application form of the composition of this invention is a powder, homogeneous powder mixture of ingredients (i)-(iv), without any auxiliary food ingredient.

In the case of common salt use (sodium chloride; NaCl) - containing yeast extracts that remains after salt-induced autolysis of yeast cell culture during their manufacturing, many commercial yeast extracts are standardized on certain percentage of salt (NaCl). If such yeast extracts are used as starting materials for manufacturing of the composition of this invention, the final product will contain certain resulting content of sodium chloride that originates from employed yeast extract(s). Of course, in the case of the use of salt-free yeast extracts, the final composition of the present invention will be essentially salt-free.

In addition to salt (NaCl), the composition of the present invention optionally comprises auxiliary food ingredients that are used either:

(i) to produce desired final form or physical state of the composition suitable for practical use as cubes, powder, granules, paste, suspension, emulsion, or solution; or

(ii) to achieve certain technological properties such as increased stability (shelf life), etc.

In that manner, the composition of the present invention optionally contains:

(i) various auxiliary food ingredients; and/or

(ii) food additives.

Auxiliary food ingredients that can be optionally employed in the composition are selected from the group comprising: maltodextrin; dextrin; starch; modified starches; inulin; oligofructose; sucrose; honey; glucose; fructose; glucose syrup; glucose-fructose syrup;

fructose-glucose syrup; cereal flours; fats and plant oils; and food grade purified water.

Fats and plant oils include all edible triglycerides such as sunflower oil, soybean oil, sesame oil, coconut oil, palm oil, hydrogenated triglycerides, other edible fats and plant oils, or mixtures of these ingredients.

Food additives that can be used in the composition of this invention are selected from the groups comprising: emulsifier, thickener, humectant, stabilizer, antioxidant, anti-caking agent, and preservative.

Emulsifier is selected from the group comprising: lecithin; hydrogenated lecithin; mono- and diglycerides of higher fatty acids like glyceryl monostearate; acetic, lactic, tartaric, and citric acid esters of mono- and diglycerides of fatty acids; esters of ethoxylated sorbitan like polysorbate 60; sucrose esters of fatty acids such as sucrose monooleate; sorbitan esters such as sorbitan monooleate; sodium or calcium stearoyl-2-lactylate; mixtures of these substances; or other food grade emulsifiers.

Thickener is selected from the group comprising: pectin; modified pectins; cellulose gums like hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), methyl cellulose (MC), or sodium carboxymethyl cellulose (NaCMC); gum arabic; xanthan gum; guar gum; carrageenan; gellan gum; karaya gum; tragacanth; cassia gum; agar; alginic acid and its salts like sodium alginate; konjac gum; locust bean gum; mixtures of these substances; or other food grade thickeners.

Humectant is selected from the group comprising: glycerine, sorbitol, mannitol, xylitol, isomalt, polydextrose, mixtures of these substances; or other food grade humectants.

Stabilizer is selected from the group comprising: sodium or potassium gluconate; glucono-delta-lactone; water soluble citrate salts like

trisodium citrate dihydrate; mixtures of these substances; or other food grade stabilizers.

Antioxidant is selected from the group comprising: ascorbic acid, its salts and esters like sodium ascorbate or ascorbyl palmitate; α-, γ-, or δ-tocopherols; mixtures of these substances; or other food grade antioxidants.

Anti-caking agent is selected from the group comprising: silicon dioxide; magnesium carbonate; talc; stearic acid; magnesium stearate; calcium silicate; mixtures of these substances; or other food grade anti-caking agents.

Preservative is selected from the group comprising: benzoic acid and its salts such as sodium benzoate; sorbic acid and its salts like potassium sorbate; sulphur dioxide generating salts like sodium sulphite or potassium metabisulphite; lactic acid; propionic acid and its salts such as calcium propionate; sodium or potassium nitrite; mixtures of these substances; or other food grade preservatives.

Preparation of the composition from the present invention

The composition of the present invention involves all application forms suitable for practical use including: cubes, powder, granules, paste, suspension, emulsion, or solution.

The form of powder is manufactured by homogenization (mixing) of:

(i) one or more yeast extracts (YEG), or hydrolysed vegetable protein (HVP), or combination thereof as a source of glutamic acid (GA);

(ii) one or more yeast extracts (YER) as a source of 5'-ribonucleotides (RN);

(iii) glutathione (GSH) or yeast extract as a source of glutathione (YEGSH);

(iv) nutritionally acceptable salt selected from: calcium salts, magnesium salts, or their combination with the weight ratio calcium : magnesium salts within the range 10:1 to 1:5; and

optionally, sodium chloride and one or more auxiliary food ingredients, e.g. anti-caking agent.

Thus obtained homogeneous powder can be granulated with small amount of suitable food grade liquid such as purified water, yielding the granulated product which is separated by sieving and dried to stable dehydrated granules.

Alternatively, homogeneous powder can be mixed with small amounts of either suitable:

(i) auxiliary food ingredients such as plant oil, honey, or glucose syrup; or

(ii) food additive like liquid sorbitol or aqueous solution of thickener; to yield a thick mass which can be pressed into the moulds furnishing the application form of cubes.

As described earlier, the term "cubes" comprises all three-dimensional generic shapes of the final product forms.

The form of the paste is prepared by homogenization of ingredients (i)-(iv) in such amount of food grade liquid to yield a pasty product. Such primary paste can be optionally stabilized with addition of emulsifier, antioxidant, stabilizer, and eventually preservative in the case of high water activity.

When higher amount of food grade liquid is used then the product is in the form of suspension as an alternative application form of the composition.

In all these cases, the food grade liquid is selected from the group of purified water and/or plant oils.

Finally, the application form of solution can be prepared by dissolution of ingredients (i)-(iv) in sufficient amount of food grade water, and eventually formulated with addition of necessary food additives like preservative, thickener, antioxidant, and stabilizer.

The preparation of all liquid forms can be performed at temperatures, from 10-100 °C, most preferably at room temperatures, e.g. at 20-25 °C.

Representative experimental preparations of the composition from this invention are described in Examples 2-18.

Study of umami and kokumi flavour enhancement characteristics of the composition from the present invention

We have found that the composition of the present invention acts as very efficient umami and kokumi taste enhancer suitable for use in savoury applications.

The methodology of the sensory testing was similar to those described in literature references 7 and 8:

7) EP1776875A1; M. Kuroda, Y. Nozawa: Seasoning composition, seasoning material and process for producing food therewith; and
8) EP2446752A1; S. Chiba: Composition for low-salt food or beverage; both of Ajinomoto Co. Inc., Japan.

The testing was performed by a six-membered panel which evaluated the following parameters or tastes: (1) saltiness; (2) umami; (3) vegetable; (4) sour; (5) sweet; and (6) overall good taste, overal deliciousness, and mouthfulness, which can be considered as kokumi taste, see literature reference 9:

9) EP0672354B1; M. Kuroda: Proteinaceous material for enhancing food taste quality; Ajinomoto Co., Inc., Japan.

The parameters were quantitatively scored from 0-4, with allowed intermediary marks 0.5, 1.5, 2.5, and 3.5; Table 2.

**Table 2**. Score criteria for sensory evaluation.

| No. | Taste intensity | Score |
|---|---|---|
| 1 | no taste | 0 |
| 2 | very weak or weak taste | 1 |
| 3 | taste of medium intensity, clear effect of respective taste | 2 |
| 4 | strong taste, strong effect of respective taste | 3 |
| 5 | very strong taste, very strong effect of respective taste | 4 |
| In the case of doubt, intermediary scores 0.5, 1.5, 2.5, and 3.5 were allowed. | | |

The testing was carried out on the matrix of model (basic) chicken-vegetable soup, whose preparation is described in Example 1. Thus prepared model soup was employed for preparation of all tested soups:

(1) a control soup; prepared by addition of salt (NaCl) and a control composition with ingredients (i)-(iii), without the calcium and/or magnesium salt (see Example 19); and

(2) a test soup; prepared from the same control soup by addition of various tested calcium and/or magnesium salt, e.g. compositions from Examples 4-8.

Since all tested compositions from Examples 4-8 do contain 27.2% NaCl originating from the starting yeast extracts YEG, YER, and YEGSH, the final test soup samples are adjusted to the same acceptable level of saltiness. Thus the amount of salt (NaCl) in all tested samples was kept constant, at the level of 0.4%, w/w.

All samples of chicken meat and vegetables are used from the same batch to avoid their variability. Also, all testings were performed at the same samples temperature of 35-45 °C. The composition of tested samples is given in Table 3.

**Table 3**. The composition of the tested samples of soups based on the formulation of the present invention without or with addition of different calcium and/or magnesium salt.

| No. | Sample | Ca/Mg salts used | Percentage of Ca/Mg salt[2] (%) |
|---|---|---|---|
| 1 | control soup [1] | - | - |
| 2 | formulation of Example 4 [3] | $CaCl_2 \bullet 2H_2O$ | 15% |
| 3 | formulation of Example 5 [3] | $MgCl_2 \bullet 6H_2O$ | 15% |
| 4 | formulation of Example 6 [3] | $CaCl_2 \bullet 2H_2O$ + $MgCl_2 \bullet 6H_2O$ | 7.5% + 7.5% |
| 5 | formulation of Example 7 [3] | Ca-lactate pentahydrate | 15% |
| 6 | formulation of Example 8 [3] | Mg-gluconate | 15% |

[1] Control soup contained: 100 ml of model soup (see Example 1), 0.10 g of additional salt (NaCl), and 1% composition of the present invention without the use of any Ca/Mg salt, see Example 19.

[2] Percentage of Ca/Mg salt within the composition of the present invention from Examples 4-8.

[3] Test soups contained: 100 g of model soup (see Example 1), 0.10 g of additional salt (NaCl), and 1% composition of the present invention with Ca/Mg salts as described in Examples 4-8.

Sensory testing gave the results of the influence of different calcium and/or magnesium salts on enhancement of umami and/or kokumi taste, as well as other tastes in the matrix of model chicken-vegetable soup. The results are presented in Table 4.

**Table 4**. The results of sensory testings of compositions of the present invention: influence of different calcium and/or magnesium salts on taste perception of umami, kokumi and other tastes.

| No. | Sample[1] | Scores of tastes[2] | | | | | | Relative intensities of tastes (±%)[3] |
|---|---|---|---|---|---|---|---|---|
| | | S | SW | V | A | U | K | |
| 1 | control | 1.00 | 0.50 | 2.00 | 0 | 1.00 | 2.00 | S: 1.00<br>SW: 1.00<br>V: 1.00<br>U: 1.00<br>K: 1.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2 | Example 4 | 1.50 | 0.50 | 2.67 | 0 | 1.67 | 2.83 | S: 1.50 (+50%)<br>V: 1.34 (+34%)<br>U: 1.67 (+67%)<br>K: 1.42 (+42%) |
| 3 | Example 5 | 1.25 | 1.08 | 2.92 | 0 | 1.67 | 2.67 | S: 1.25 (+25%)<br>SW: 2.16 (+116%)<br>V: 1.46 (+46%)<br>U: 1.67 (+67%)<br>K: 1.34 (+34%) |
| 4 | Example 6 | 1.50 | 1.00 | 2.67 | 0 | 1.75 | 2.92 | S: 1.50 (+50%)<br>SW: 2.00 (+100%)<br>V: 1.34 (+34%)<br>U: 1.75 (+75%)<br>K: 1.46 (+46%) |
| 5 | Example 7 | 1.17 | 0.92 | 2.67 | 0 | 1.67 | 2.75 | S: 2.34 (+134%)<br>SW: 1.84 (+84%)<br>V: 1.34 (+34%)<br>U: 1.67 (+67%)<br>K: 1.38 (+38%) |
| 6 | Example 8 | 1.00 | 1.42 | 2.67 | 0 | 2.00 | 2.08 | SW: 2.84 (+184%)<br>V: 1.34 (+34%)<br>U: 2.00 (+100%)<br>K: 1.04 (+4%) |

[1] In the preparation of corresponding samples of tested soups, either blank composition of the present invention without Ca/Mg salts from Example 19, or compositions from Examples 4-8 with different Ca and/or Mg salts were employed.

[2] Tastes: S= salty, SW= sweet, V= vegetable, A= sour, acidic, U= umami, K= overal deliciousness, mouthfulness, kokumi.

[3] Relative intensities are calculated as a ratio of the respective taste score of given sample versus the score of the control soup. Represent a degree of decreasing or increasing of perception of the given taste.

These results clearly show that the blank control formulation from Example 19, containing only:

(i) yeast extract (YEG) based on natural glutamic acid (GA);

(ii) yeast extract (YER) based on natural 5'-ribonucleotides (RN); and

(iii) yeast extract (YEGSH) based on natural glutathione (GSH); without any calcium and/or magnesium salt, exhibits clearly inferior umami and kokumi tastes perception than samples based on the composition of the present invention that contain Ca/Mg salts, Examples 4-8.

In this manner, it is clear that calcium/magnesium salt of nutritionally acceptable acids such as calcium chloride, magnesium chloride, calcium lactate, magnesium gluconate, etc., do act as effective agent that enhance umami and kokumi tastes perception of the base formulation of yeast extracts (i) + (ii) + (iii). This indicates a synergic action of calcium and/or magnesium salts on enhancement of umami and kokumi tastes perception of combination of ingredients (i) + (ii) + (iii).

Beside umami and kokumi tastes enhancement, certain calcium and/or magnesium salts do exhibit certain degree of enhancement of other also very useful tastes, such as:

(i) calcium and/or magnesium chloride for salty taste; and

(ii) all magnesium salts for sweet taste and vegetable notes perception.

Additionally, it was observed that the combination of calcium + magnesium salts provides slightly better umami taste perception than the same parent salt used alone. However this cannot be considered as fully established correlation, but only as an indication of possible additional synergistic relationship of calcium + magnesium salts combination on enhancement of umami taste perception of basic ingredients of this composition: (i) + (ii) + (iii).

Further study was focussed on initially indicated synergistic action of calcium and magnesium salts on enhancement of umami and kokumi tastes perception of the combination of:

(i) yeast extract (YEG) based on natural glutamic acid (GA);

(ii) yeast extract (YER) based on natural 5'-ribonucleotides (RN); and

(iii) yeast extract (YEGSH) based on natural glutathione (GSH).

To get further supports of this indication, we prepared alternative composition of this invention as described in Example 9, which contained:

(i) yeast extract (YEG) based on natural glutamic acid (GA);

(ii) yeast extract (YER) based on natural 5'-ribonucleotides (RN);
(iii) yeast extract (YEGSH) based on natural glutathione (GSH); and
(iv) one specific combination of Ca- and Mg-salts, calcium lactate and magnesium gluconate.

The composition from Example 9 was further subjected to sensory testing against the control composition that contained ingredients (i), (ii), and (iv) only, and which was totally without ingredient (iii) - the yeast extract (YEGSH) based on natural glutathione (GSH); see Example 20. In the same time, the control composition was filled with (i) instead of (iii) up to 100% of the formulation.

In this manner, if the synergy of (iii) and (iv) really exists, then significant improvement of the composition of Example 9 would be expected over the control composition (Example 20) that contains only Ca/Mg salts without the GSH (Table 5).

**Table 5.** The composition of control formulation against the model fomulation from the present invention for additional study of synergistic action of calcium and/or magnesium salts and glutathione (GSH) on enhancement of umami and kokumi tastes perception of yeast extracts based on glutamic acid (YEG) and ribonucleotides (YER).

| No. | Ingredient (IN) | Control (example 20) / Example 9 [1] | | |
|---|---|---|---|---|
| | | Composition (%)[1] | Content of NaCl (%)[2] | Content of NaCl (%)[3] |
| 1 | YEG: Ohly M1T[4] | 60 / 40 | 40 | 24 / 16 |
| 2 | YER: Aromild[5] | 20 / 20 | 10 | 2 / 2 |
| 3 | YEGSH: High Lyfe 610A[6] | - / 20 | 2 | - / 0.4 |
| 4 | Ca-lactate[7] | 10 / 10 | - | - |
| 5 | Mg-gluconate | 10 / 10 | - | - |
| | **Total:** | 100 / 100 | - | 26 / 18.4 [8] |

[1] Composition of the formulation of the present invention from Example 9 and its control composition without GSH-containing ingredient from Example 20;

[2] Content of salt (NaCl) in each type of commercially available yeast extract;

[3] Contents of NaCl in final composition that originate from respective type of yeast extract;

[4] Product of Ohly GmbH, Germany;

[5] Product of Kohjin LifeSciences Co. Inc., Japan;

[6] Product of Lallemand, Canada;

[7] Calcium lactate pentahydrate; and

[8] Total content of salt (NaCl) in the compositions from Examples 9 and 20; this was corrected to the same level with additional NaCl during the sensory testing.

Due to strong influence of salt (NaCl) on overall taste perception, as well as different content of NaCl in the composition of the present invention from Example 9 and its control composition from Example 20, the total content of NaCl in all tested soups samples were corrected to the same level to avoid potential source of mistake.

Such prepared composition (Example 9) and its control formulation (Example 20) were used for preparation of test soups, whose compositions are described in Table 6.

**Table 6.** The compositions of tested sample soups for sensory testings of the formulation from the present invention from Example 9 and its control formulation from Example 20.

| No. | Ingredient | Control (Example 20)[1] | The composition (Example 9)[1] |
|---|---|---|---|
| 1 | control composition of Example 20 (26% NaCl) | **0.81 g** | - |
| 2 | composition of Example 9 (18.4% NaCl) | - | 0.74 g |
| 3 | salt (NaCl) | 0.19 g[2] | 0.27 g[2] |
| 4 | model soup (see Example 1) | 100 ml | 100 ml |

[1] The content of total yeast extracts in both tested soups was the same: 0.6% w/w.

[2] The content of salt (NaCl) in both tested soups was the same: 0.4% w/w.

Sensory testing was performed by the same methodology as described in Example 19. The results of sensory testing are given in Table 7.

**Table 7**. The results of sensory testing of compositions of the present invention from Example 9 against its control formulation without the GSH-containing yeast extract: synergistic effect of calcium and magnesium salts + GSH on taste perception of umami, kokumi and other tastes.

| No. | Sample[1] | Scores of tastes[2] | | | | | | Relative intensities of taste (±%)[3] |
|---|---|---|---|---|---|---|---|---|
| | | S | SW | V | A | U | K | |
| 1 | control, Example 20 | 1.17 | 0.83 | 2.33 | 0 | 1.50 | 2.50 | U: 1.00<br>K: 1.00 |
| 2 | composition, Example 9 | 1.25 | 1.00 | 2.83 | 0 | 2.17 | 3.00 | U: 1.45 (+45%)<br>K: 1.20 (+20%) |

[1] In the preparation of corresponding samples of tested soups, either blank composition of the present invention without GSH from Example 19, or composition from Example 9 with GSH, were employed;

[2] Tastes: S= salty, SW= sweet, V= vegetable, A= sour, acidic, U= umami, K= overall deliciousness, mouthfulness, kokumi; and

[3] Relative intensities are calculated as a ratio of the respective taste score of given sample versus the score of the control soup. Represent a degree of decreasing or increasing of perception of the given taste.

Once again, obtained results clearly suggest synergistic action of:

(a) calcium/magnesium salts, herein a combination of calcium lactate and magnesium gluconate, and

(b) glutathione (GSH),

on enhancement of umami (+45%) and kokumi (+20%) tastes perception of the model chicken-vegetable soup prepared with a combination of yeast extracts based on glutamic acid (YEG), ribonucleotides (YER) and calcium and magnesium salts.

The formulations (Examples 4-8,9) that contained both GSH and Ca-/Mg-salts gave better results than the formulations that, beside yeast extracts YEG, YER as sources of glutamic acid (GA) and 5'-ribonucleotides (RN), (i+ii), contained:

1. either GSH (control, Example 19) only;
2. or Ca-/Mg-salts (control, Example 20) only.

In conclusion, calcium and/or magnesium salts in the presence of glutathione (GSH) or glutathione-based yeast extracts (YEGSH) do act, presumably in a synergistic manner, on enhancement of umami and kokumi tastes perception of basic formulation of:

(i) yeast extract (YEG) based on natural glutamic acid (GA);

(ii) yeast extract (YER) based on natural 5'-ribonucleotides (RN);

whose (i) + (ii) umami-enhancing properties are known from the previous art.

The use of the composition of taste or flavour enhancer from the invention

The composition of the present invention acts as efficient umami and kokumi taste enhancer which is suitable for use in savoury applications. The composition can be used as sole seasoning agent, as table-top seasoning.

Additionally the composition is employed as industrial product, meaning as taste or flavour enhancing agent intended for use in manufacturing of food products providing umami and kokumi flavours enhancement.

The food product wherein the composition can be used as taste enhancer is taken from the group consisting of:

(i) culinary products such as soups, bouillons, food seasonings, and sauces;

(ii) dry foods including snacks, cereals, and biscuits;

(iii) meat products including ham, sausages, and pates;

(iv) milk products such as processed milk or fermented milk products;

(v) bakery products including bread and baked confections;

(vi) fats and processed oily foods like salad oils, margarine, butter, mayonnaise, salad dressings, and shortenings;

(vii) marine products;

(viii) prepared meals like frozen, sterilized, or chilled products and foodservice products;

(ix) nutritional products including foods for special nutritional purposes;

(x) flavours and flavour ingredients;

(xi) beverages;

(xii) animal feed; or

(xiii) any other food product wherein glutamate or other umami-taste providing ingredient is part of the composition.

Typically, the composition is used in amounts between 0.01-50%, w/w, of the final food product depending on the formulation thereof.

**Examples**

General remarks

All quantitative compositions are given in weight (w/w) percentages (%). Yeast extracts (YE), deactivated yeasts (DY), and hydrolyzed vegetable proteins (HVP) were purchased from manufacturers as described in footnotes of the Tables 1 and 8, and in experimental examples. Food grade calcium and magnesium salts were purchased from Dr. Paul Lohmann GmbH KG, Germany, and Purac NV, The Netherlands, unless otherwise noted. Room temperature (r.t.) means the temperature interval of 20-25 °C.

Example 1. Sensory evaluation of yeast extracts (YE), deactivated yeasts (DY), and hydrolyzed vegetable proteins (HVP) that are suitable for production of the composition from the present invention

The sensory evaluation of various commercially available yeast extracts (YE), deactivated yeasts (DY), and hydrolyzed vegetable proteins (HVP) were performed in the model chicken-vegetable soup matrix by the methodology similar to those described in literature references 7 and 8, whose scoring was explained in the Table 2. The samples were tested against blank model soup as the control (standard).

Preparation of the model chicken-vegetable soup: this was prepared from chicken wings (150 g) and sliced vegetables: onion (50 g), celery

root (50 g), and carrot (100 g). The mixture was cooked in mildly boiling water (1000 ml) for 1 h. Then, the soup was allowed to cool to 50-60 °C, and decanted to remove all meat and vegetables. Total volume of thus obtained decantate was adjusted to 1000 ml with addition of warm pot water. Thus obtained product was used as a model soup for sensory testing.

All samples of chicken meat and vegetables are used from the same batch to avoid their variability.

Preparation of sample soups with different YE, DY, and HVP: to model chicken-vegetable soup (100 ml) tested YE, DY, or HVP was added in such amount to yield 0.60 g of pure, salt-free YE, DY, or HVP, and 0.40 g of total salt (NaCl). The testing temperature was 35-45 °C.

The results of sensory testing are described in Table 8.

**Table 8.** The results of sensory testing of various commercially available yeast extracts (YE), deactivated yeasts (DY), and hydrolyzed vegetable proteins (HVP) in a matrix of the model chicken-vegetable soup.

| No. | YE (or DY or HVP) | Specification | Umami | Overall deliciousness |
|---|---|---|---|---|
| 1 | - (standard; STD)[1] | - | 1.00 (= STD) | 1.00 (= STD) |
| 2 | Aromild[2] | 10% NaCl, 6% N, 20% IMP/GMP | 3.50 (350%) | 3.42 (342%) |
| 3 | Umamex 100LS[3] | 0.5% NaCl, 6% IMP/GMP | 3.33 (333%) | 3.33 (333%) |
| 4 | Ohly STT[4] | 40% NaCl, 45% protein | 3.25 (325%) | 3.00 (300%) |
| 5 | Ohly M1T[4] | 40% NaCl, 40% protein | 3.00 (300%) | 3.00 (300%) |
| 6 | Gistex LS powder[5] | 1% NaCl, 10% N | 3.17 (317%) | 2.83 (283%) |
| 7 | High Lyfe 560A[6] | 2% NaCl, 53% protein, 14% IMP/GMP | 2.25 (225%) | 2.75 (275%) |
| 8 | Ohly FLAV-A-ROUND LS[4] | 1% NaCl, 70-75% protein | 2.17 (217%) | 2.50 (250%) |
| 9 | Springer 4115/ 0-MG-L[7] | 0% NaCl, 9.5% N, 2.83% amino N | 167 (167%) | 2.42 (242%) |

| | | | | |
|---|---|---|---|---|
| 10 | Pronal 5001/ 0-PW-L[7] | 1% NaCl, 60% protein | 167 (167%) | 2.33 (232%) |
| 11 | Springer 2012/ 20-MG-L[7] | 20% NaCl, 45% protein | 2.00 (200%) | 2.25 (225%) |
| 12 | HVP Vitana[8] | 42-50% NaCl, 26-34% protein, 7-11% natural MSG | 1.67 (167%) | 2.25 (225%) |
| 13 | High Lyfe 610A[6] | 2% NaCl, 45% protein, 10% GSH | 2.00 (200%) | 2.08 (208%) |
| 14 | Leiber Bouillon HR[9] | 34-38% NaCl, 42-52% protein, 3% GMP/IMP | 1.92 (192%) | 2.08 (208%) |
| 15 | Leiber Bouillon G[9] | 36-40% NaCl, 40-50% protein, 4% natural MSG | 1.50 (150%) | 2.00 (200%) |
| 16 | Leiber Bouillon N[9] | 35-41% NaCl, 39-49% protein | 1.42 (142%) | 2.00 (200%) |
| 17 | Springer 2006/ 0-MG-L[7] | 1% NaCl, 10-12.4% N, 5-7% GMP/IMP | 1.33 (133%) | 2.00 (200%) |
| 18 | Multirome LS Powder[5] | 10% NaCl, 7% N | 3.00 (300%) | 1.92 (192%) |
| 19 | High Lyfe 608A[6] | 2% NaCl, 60% protein, 8% GSH | 2.25 (225%) | 1.83 (183%) |
| 20 | Maxarome Select Powder AGGL[5] | 20% NaCl, 12% N, 14-16% GMP/IMP | 3.33 (333%) | 1.67 (167%) |
| 21 | Ohly BOT LS[4] | 3% NaCl, 65% protein | 2.08 (208%) | 1.67 (167%) |
| 22 | Springer 2020/ 0-MG-L[7] | 1% NaCl, 9.5% N, 60% protein | 1.58 (158%) | 1.67 (167%) |
| 23 | DY Springalys D100/0-PW[7] | 0% NaCl, 9.5% N, 60% protein | 2.50 (250%) | 1.50 (150%) |
| 24 | Gistex Standard Powder AGGL[5] | 40% NaCl, 40% protein | 2.33 (233%) | 1.50 (150%) |
| 25 | HVP Nutralys W[10] | 0% NaCl, 80-85% protein | 1.00 = STD | 1.17 (117%) |

[1] In this run model chicken-vegetable soup without any YE, DY, or HVP was used;

[2] Product of Kohjin LifeScience Co. Ltd., Japan;

[3] Product of Kerry Group plc, Ireland;

[4] Product of Ohly GmbH, Germany;

[5] Product of DSM Nutritional Products Ltd, Switzerland;

[6] Product of Lallemand Inc., Canada;

[7] Product of Bio Springer, France;

[8] Product of Rieber & Søn ASA, Norway/Czech Republic;

[9] Product of Leiber GmbH, Germany; and

[10] Product of Roquette, France.

This testing showed that numerous commercially available yeast extracts (YE) of various types, hydrolyzed vegetable proteins (HVP), and even deactivated yeasts (DY) can be employed as basic ingredients in the formulation of the present invention as all of them clearly improve umami taste perception, as well as overall deliciousness of chicken-vegetable model soup.

These ingredients are used according to manufacturers declarations as either sources of natural glutamic acid (GA), ribonucleotides (RN; IMP, GMP), or glutathione (GSH). Various other commercially available yeast extracts (YE), deactivated yeasts (DY), and hydrolyzed vegetable proteins (HVP) can be used as sources of these key natural ingredients of the composition of this invention.

Example 2. Preparation of the composition of the invention in the form of powder with 0.01% glutathione (GSH) and 5% calcium chloride dihydrate

Composition (for 100 g of powder):

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(1) 75.00 g (75.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 12.50 g (12.50%) yeast extract (YER); type Umamex 100LS[2]
(3) 0.02 g (0.02%) food grade 50% glutathione (GSH), reduced[3]
(4) 5.00 g (5.00%) calcium chloride dihydrate, food grade
(5) 6.48 g (6.48%) maltodextrin; type Glucidex 39[4]
(6) 1.00 g (1.00%) magnesium carbonate, light, food grade

---

Preparation: GSH was added to maltodextrin. The mixture was homogenized in V-blender for 15 minutes. Thus obtained glutathione premix was added to the mixture of YEG, YER, and calcium chloride dihydrate. The mixture was homogenized in V-blender for 15 minutes. Then magnesium carbonate was added, and further homogenized for 5 minutes. The product was in the form of pale brown flowable powder; composition: 0.01% GSH, 1.3% calcium (Ca).

[1] Product of Ohly GmbH, Germany;

[2] Product of Kerry Group plc, Ireland;

[3] Product of Beckmann-Kenko GmbH, Germany; and

[4] Product of Roquette, France.

Example 3. Preparation of the composition of the invention in the form of granules with 3% glutathione and 3% magnesium chloride hexahydrate

Composition (for 100 g of granules):

---

(1) 5.00 g (5.00%) yeast extract (YEG); type Leiber BOUILLON G[1]

(2) 75.00 g (75.00%) yeast extract (YER); type Aromild[2]

(3) 6.00 g (6.00%) food grade 50% glutathione (GSH), reduced[3]

(4) 3.00 g (3.00%) magnesium chloride hexahydrate, food grade

(5) 10.00 g (10.00%) oligofructose; type Frutalose OFP[4]

(6) 1.00 g (1.00%) colloidal silicon dioxide; type Aerosil 200F[5]

---

Preparation: GSH was added to oligofructose. The mixture was homogenized in V-blender for 10 minutes. Thus obtained glutathione premix was added to the mixture of YEG, YER, and magnesium chloride hexahydrate. Resulting mixture was homogenized in V-blender for 10 minutes. Then silicon dioxide was added, and further homogenized for 5 minutes.

To such homogeneous mixture, purified water (5.00 g) was added, and the mixture was granulated, subsequently forced through a sieve, and dried in a vacuum oven at 40 °C for 20 hours.

The product was in the form of yellowish to pale brown granules; composition: 3.00% GSH, 0.35% magnesium (Mg).

[1] Product of Leiber GmbH, Germany;

[2] Product of Kohjin LifeScience Co. Ltd., Japan;

[3] Product of Beckmann-Kenko GmbH, Germany;

[4] Product of Sensus, The Netherlands; and

[5] Product of Evonik Industries AG, Germany.

Example 4. Preparation of the composition of the invention in the form of powder with 1% glutathione and 15% calcium chloride

Composition (for 100 g of powder):

---

(1) 65.00 g (65.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 10.00 g (10.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 15.00 g (15.00%) calcium chloride dihydrate, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to pale brown powder; composition: 1.00% GSH, 4.00% calcium (Ca), 27.2% salt (NaCl).

[1] Product of Ohly GmbH, Germany;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Example 5. Preparation of the composition of the invention in the form of powder with 1% glutathione and 15% magnesium chloride

Composition (for 100 g of powder):

---

(1) 65.00 g (65.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 10.00 g (10.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 15.00 g (15.00%) magnesium chloride hexahydrate, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes.
The product was in the form of yellowish to pale brown powder; composition: 1.00% GSH, 1.75% magnesium (Mg), 27.2% salt (NaCl).

[1] Product of Ohly GmbH, Germany;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Example 6. Preparation of the composition of the invention in the form of powder with 1% glutathione, 7.5% calcium chloride and 7.5% magnesium chloride

Composition (for 100 g of powder):

---

(1) 65.00 g (65.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 10.00 g (10.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 7.50 g (7.50%) calcium chloride dihydrate, food grade
(5) 7.50 g (7.50%) magnesium chloride hexahydrate, food grade

---

Preparation: The mixture of (1)-(5) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to pale brown powder; composition: 1.00% GSH, 2.00% calcium (Ca), 0.88% magnesium (Mg), 27.2% salt (NaCl).

[1] Product of Ohly GmbH, Germany;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Example 7. Preparation of the composition of the invention in the form of powder with 1% glutathione and 15% calcium lactate

Composition (for 100 g of powder):

---

(1) 65.00 g (65.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 10.00 g (10.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 15.00 g (15.00%) calcium lactate pentahydrate, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to pale brown powder; composition: 1.00% GSH, 1.95% calcium (Ca), 27.2% salt (NaCl).

[1] Product of Ohly GmbH, Germany;

[2] Product of Kohjin LifeScience Co. Ltd., Japan;

[3] Product of Lallemand Inc., Canada.

Example 8. Preparation of the composition of the invention in the form of powder with 1% glutathione and 15% magnesium gluconate

Composition (for 100 g of powder):

---

(1) 65.00 g (65.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 10.00 g (10.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 15.00 g (15.00%) magnesium gluconate, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to pale brown powder; composition: 1.00% GSH, 0.86% magnesium (Mg), 27.2% salt (NaCl).

[1] Product of Ohly GmbH, Germany;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Example 9. Preparation of the composition of the invention in the form of powder with 2% glutathione, 10% calcium lactate and 10% magnesium gluconate

Composition (for 100 g of powder):

---

(1) 40.00 g (40.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 20.00 g (20.00%) yeast extract (YER); type Aromild[2]
(3) 20.00 g (20.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 10.00 g (10.00%) calcium lactate pentahydrate, food grade
(5) 10.00 g (10.00%) magnesium gluconate, food grade

---

Preparation: The mixture of (1)-(5) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to

pale brown powder; composition: 2.00% GSH, 1.30% calcium (Ca), 0.57% magnesium (Mg), 18.4% salt (NaCl).

[1] Product of Ohly GmbH, Germany;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Example 10. Preparation of the composition of the invention in the form of powder with 1% glutathione and 1% calcium citrate

Composition (for 100 g of powder):

---

(1) 68.00 g (68.00%) hydrolysed vegetable protein (HVP); type Vitana[1]
(2) 20.00 g (20.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 1.00 g (1.00%) calcium citrate, food grade
(5) 1.00 g (1.00%) magnesium carbonate, light, food grade

---

Preparation: The mixture of (1)-(5) was placed into the V-blender and homogenized 15 minutes. The product was in the form of pale brown powder; composition: 1.00% GSH, 0.24% calcium (Ca).

[1] Product of Rieber & Søn ASA, Norway/Czech Republic;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Example 11. Preparation of the composition of the invention in the form of granules with 1% glutathione, 4.84% calcium citrate tetrahydrate and 4.5% magnesium hydrogencitrate

Composition (for 100 g of granules):

---

(1) 45.66 g (45.66%) yeast extract (YEG); type Leiber BOUILLON G[1]
(2) 25.00 g (25.00%) yeast extract (YER); type Leiber Aromor GS20[1]
(3) 20.00 g (20.00%) yeast extract (YEGSH); type High Lyfe 605A[2]
(4) 4.84 g (4.84%) calcium citrate tetrahydrate, food grade
(5) 4.50 g (4.50%) magnesium hydrogencitrate, food grade

---

Preparation: The mixture of (1)-(5) was homogenized in V-blender for 15 minutes. To such homogeneous mixture, purified water (5.00 g) was added, and the mixture was granulated, subsequently forced through a sieve, and dried in a vacuum oven at 40 °C overnight. The product was in the form of pale brown granules; composition: 1.00% GSH, 1.00% calcium (Ca), 0.5% magnesium (Mg).

[1] Product of Leiber GmbH, Germany; and

[2] Product of Lallemand Inc., Canada.

Example 12. Preparation of the composition of the invention in the form of paste with 0.5% glutathione and 1% magnesium sulfate heptahydrate

Composition (for 100 g of paste):

---

(1) 45.00 g (45.00%) yeast extract (YEG); type Springer 4115/0-MG-L[1]
(2) 20.00 g (20.00%) yeast extract (YER); type High Lyfe 560A[2]
(3) 5.00 g (5.00%) yeast extract (YEGSH); type High Lyfe 610A[2]
(4) 1.03 g (1.03%) magnesium sulphate heptahydrate, food grade
(5) 3.00 g (3.00%) polysorbate 60, food grade
(6) 25.97 g (25.97%) canola oil, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. Thus obtained homogeneous powder mixture was milled and then added to previously prepared mixture of canola oil and polysorbate 60 with high speed stirring to yield a paste. The product was in the form of yellowish to light brown paste; composition: 0.50% GSH, 0.10% magnesium (Mg).

[1] Product of Bio Springer, France; and

[2] Product of Lallemand Inc., Canada.

Example 13. Preparation of the composition of the invention in the form of suspension with 1% glutathione, 5.7% of calcium gluconate and 5% magnesium lactate

Composition (for 100 g of suspension):

---

(1) 30.00 g (30.00%) yeast extract (YEG); type Ohly STT[1]
(2) 15.00 g (15.00%) yeast extract (YER); type Umamex 100LS[2]
(3) 6.67 g (6.67%) yeast extract (YEGSH); type Aromild U-15[3]
(4) 5.71 g (5.71%) calcium gluconate monohydrate, food grade
(5) 5.00 g (5.00%) magnesium lactate dihydrate, food grade
(6) 0.30 g (0.30%) xanthan gum FN type[4]
(7) 0.20 g (0.20%) sodium benzoate, food grade
(8) 37.12 g (37.12%) purified water, food grade

---

Preparation: To purified water (8), xanthan gum (6) and sodium benzoate (7) were added and dissolved with stirring at room temperature for 15 minutes. Then, ingredients (1)-(5) were added with vigorous stirring. The resulting mixture was heated to 40-45 °C and homogenized for 30 minutes at high speed stirring, and subsequently cooled to room temperature. The product was in the form of yellowish to light brown viscous suspension; composition: 1.00% GSH, 0.50% calcium (Ca), 0.50% magnesium (Mg).

[1] Products of Ohly GmbH, Germany;
[2] Product of Kerry Group plc, Ireland;
[3] Product of Kohjin LifeScience Co. Ltd., Japan; and
[4] Product of Jungbunzlauer AG, Switzerland.

Example 14. Preparation of the composition of the invention in the form of solution with 0.5% glutathione and calcium containing mineral water

Composition (for 100 g of solution):

---

(1) 20.00 g (20.00%) yeast extract (YEG); type Springer 2006/0-MG-L[1]
(2) 5.00 g (5.00%) yeast extract (YER); type Umamex 100LS[2]
(3) 1.00 g (1.00%) yeast extract (YEGSH), 50% GSH[3]
(4) 74.00 g (74.00%) calcium-containing mineral water Studena[4]

---

Preparation: To mineral, calcium-containing water, ingredients (1)-(3) were added and the resulting mixture was heated to 40-45 °C and dissolved for 30 minutes with stirring. The product was subsequently cooled to room temperature and filtered. The product was in the form of yellowish to light brown solution; composition: 0.50% GSH, 0.005-0.006% calcium (Ca).

[1] Products of Bio Springer, France;
[2] Product of Kerry Group plc, Ireland;
[3] Product of Beckmann-Kenko GmbH, Germany; and
[4] Product of Podravka d.d., Croatia.

Example 15. Preparation of the composition of the invention in the form of powder with 10% glutathione and 3.74% calcium chloride

Composition (for 100 g of powder):

---

(1) 45.26 g (45.26%) yeast extract (YEG); type Springer 2012/20-MG-L[1]
(2) 30.00 g (30.00%) yeast extract (YER); type Leiber AROMOR GS20[2]
(3) 20.00 g (20.00%) yeast extract (YEGSH), 50% GSH[3]
(4) 3.74 g (3.74%) calcium chloride dihydrate, food grade
(5) 1.00 g (1.00%) magnesium carbonate, light, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. Then (5) was added and homogenized for additional 5 minutes. The product was in the form of pale brown powder; composition: 10.00% GSH, 1.00% calcium (Ca).

[1] Product of Bio Springer, France;
[2] Product of Leiber GmbH, Germany; and
[3] Product of Beckmann-Kenko GmbH, Germany.

Example 16. Preparation of the composition of the invention in the form of cubes with 1% glutathione and 50% calcium-containing milk permeate

Composition (for 100 g of mass for pressing cubes):

---

(1) 13.00 g (13.00%) yeast extract (YEG); type Leiber BOUILLON N[1]

(2) 10.00 g (10.00%) yeast extract (YER); type Leiber AROMOR GS20[1]

(3) 2.00 g (2.00%) yeast extract (YEGSH), 50% GSH[2]

(4) 50.00 g (50.00%) calcium-containing milk permeate powder, food grade

(5) 25.00 g (25.00%) coconut oil, food grade

---

Preparation: Coconut oil was heated to 40-45 °C and then ingredients (1)-(4) were gradually added during 15 minutes with constant stirring. Thus obtained thick mass was cooled to room temperature and pressed into cube moulds. The product was in the form of yellowish to light brown cubes; composition: 1.00% GSH, 0.30% calcium (Ca), min. 40.00% lactose.

[1] Product of Leiber GmbH, Germany; and

[2] Product of Beckmann-Kenko GmbH, Germany.

Example 17. Preparation of the composition of the invention in the form of powder with 2% glutathione and 30% calcium caseinate

Composition (for 100 g of powder):

---

(1) 50.00 g (50.00%) yeast extract (YEG); type Springer 4115/0-MG-L[1]

(2) 15.00 g (15.00%) yeast extract (YER); type Leiber AROMOR GS20[2]

(3) 4.00 g (4.00%) yeast extract (YEGSH), 50% GSH[3]

(4) 30.00 g (30.00%) calcium caseinate; food grade

(5) 1.00 g (1.00%) magnesium carbonate, light, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. Then (5) was added and homogenized for additional 5 minutes. The product was in the form of yellowish to light brown powder; composition: 2.00% GSH, 0.40% calcium (Ca).

[1] Product of Bio Springer, France;

[2] Product of Leiber GmbH, Germany; and

[3] Product of Beckmann-Kenko GmbH, Germany.

Example 18. Preparation of the composition of the invention in the form of powder with 1% glutathione and 5% Dead Sea salt

Composition (for 100 g of powder):

---

(1) 54.00 g (54.00%) yeast extract (YEG); type Springer 4115/0-MG-L[1]
(2) 20.00 g (20.00%) yeast extract (YER); type Umamex 100LS[2]
(3) 20.00 g (20.00%) yeast extract (YEGSH); type High Lyfe 605A[3]
(4) 5.00 g (5.00%) bromide-free Dead Sea salt
(5) 1.00 g (1.00%) magnesium carbonate, light, food grade

---

Preparation: Bromide-free Dead Sea salt was milled to the particles size of 0.1-0.3 mm (>90%). Then, such milled (4) was placed together with ingredients (1)-(3) into the V-blender, and homogenized for 15 minutes. Then, ingredient (5) was added and additionally homogenized for 3 minutes. The product was in the form of yellowish to light brown powder; composition: 1.00% GSH, 0.40% magnesium (Mg).

[1] Product of Bio Springer, France;
[2] Product of Kerry Group plc, Ireland; and
[3] Product of Lallemand, Canada.

Example 19. Sensory evaluation of the composition of the present invention. Influence of various calcium and magnesium salts.

Sensory evaluation of the composition of the present invention was performed on a model chicken-vegetable soup by the similar methodology as described in the literature references 7 and 8. Preparation of the model soup is described in Example 1.

Thus prepared model soup was employed for preparation of all tested samples soups:

(1) a control soup; prepared by addition of salt (NaCl) and a control composition with ingredients (i)-(iii), without the calcium and/or magnesium salt; and

(2) a test soup; prepared from the same control soup by addition of tested calcium and/or magnesium salt, e.g. compositions from Examples 4-8.

The control soup without the Ca/Mg-salts was prepared by the use of the control taste enhancer of the same formulation as the compositions from the present invention described in Examples 4-8, but without the use of any Ca/Mg-salt.

Preparation of control taste enhancer based on yeast extracts (i-iii) without Ca/Mg-salts:

Control taste enhancer composition (for 100 g of powder):

---

(1) 65.00 g (65.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 10.00 g (10.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) yeast extract (YEGSH); type High Lyfe 610A[3]
(4) 15.00 g (15.00%) cornstarch, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to pale brown powder; composition: 1.00% GSH, 27.2% salt (NaCl).

[1] Product of Ohly GmbH, Germany;
[2] Product of Kohjin LifeScience Co. Ltd., Japan; and
[3] Product of Lallemand Inc., Canada.

Such obtained control taste enhancer and compositions from the present invention with different calcium and/or magnesium salts were used for preparation of test soups samples. Contents of yeast extracts, glutathione, and salt (NaCl) in the control and test soups samples were kept the same at:

(i) 0.6% of total yeast extracts (YE);
(ii) 0.006% of glutathione (GSH); and
(iii) 0.4% of total salt (NaCl).

Preparation of the control soups: to a model soup (100 ml; see Example 1), control taste enhancer up to 0.6% of total yeast extracts content, and additional salt up to total content of 0.4% NaCl, in the final sample were added. The mixture was stirred for 5 minutes and served at 35-45 °C.

Preparation of the test soups: the same as control soup, except that instead of the control taste enhancer, compositions of the present invention from Examples 4-8 were used at total yeast extract content of 0.6% and salt content of 0.4%. The compositions of tested samples soups are given in Table 3.

The testing was performed by a six-membered panel which evaluated the following tastes: (1) saltiness; (2) umami; (3) vegetable; (4) sour; (5) sweet; and (6) good taste, overall deliciousness and mouthfulness, which can be considered as kokumi taste, see the literature reference 3. The tastes were quantitatively scored from 0-4, with allowed intermediary marks 0.5, 1.5, 2.5, and 3.5, as described in Table 2.

Since all tested compositions from Examples 4-8 contained 27.2% NaCl originating from the starting yeast extracts YEG, YER, and YEGSH, the final test soup samples are adjusted to acceptable level of saltines. Thus the amount of salt (NaCl) in all tested samples was kept constant, at 0.4%, m/m. All testings were performed at temperature samples of 35-45 °C.

Sensory testing gave the result of the influence of different calcium and/or magnesium salts on enhancement of umami and/or kokumi taste, as well as other tastes in the matrix of model chicken-vegetable soup. The results are described in Table 4.

Example 20. Sensory evaluation of the composition of the present invention. Additional proof for synergy of taste enhancement by the combination of glutathione with calcium and magnesium salts.

The composition from Example 9 was subjected to sensory testing against the control composition that contained only the ingredients:

(1) yeast extract (YEG) based on natural glutamic acid (GA);
(2) yeast extract (YER) based on natural 5'-ribonucleotides (RN); and
(3) calcium and/or magnesium salts; totally without the yeast extract (YEGSH) based on natural glutathione (GSH).

This control formulation without GSH was, instead of glutathione (GSH)-containing yeast extract (YEGSH), filled to up to 100% w/w with the basic yeast extract (YEG). Its preparation is as follows:

Composition (for 100 g of powder):

---

(1) 60.00 g (60.00%) yeast extract (YEG); type Ohly M1T[1]
(2) 20.00 g (20.00%) yeast extract (YER); type Aromild[2]
(3) 10.00 g (10.00%) calcium lactate pentahydrate, food grade
(4) 10.00 g (10.00%) magnesium gluconate, food grade

---

Preparation: The mixture of (1)-(4) was placed into the V-blender and homogenized 15 minutes. The product was in the form of yellowish to pale brown powder; composition: 1.30% calcium (Ca), 0.57% magnesium (Mg), 26% salt (NaCl).

[1] Product of Ohly GmbH, Germany; and
[2] Product of Kohjin LifeScience Co. Ltd., Japan.

Such prepared control taste enhancer without GSH, together with the test composition of the present invention from Example 9, were used in the preparation of test soups samples, whose compositions are described in Table 6. Once again, the total yeast extract and total salt contents were kept the same at optimal level of 0.6% and 0.4%.

The sensory testing was carried out by the same methodology as described in Example 19. The results are given in Table 7.

**Industrial Applicability**

The composition from the present invention is used for manufacturing of taste or flavour enhancer that is employed either as sole seasoning

agent or as food ingredient in various savoury applications. Therefore, industrial applicability of this invention is obvious.

**CLAIMS**

1. A composition of umami and kokumi taste enhancer suitable for practical use in the form of: cubes, powder, granules, paste, suspension, emulsion, or solution; where said composition having ingredients (i) and (ii):

   (i) one or more yeast extracts, or hydrolysed vegetable protein, or combination thereof as a source of glutamic acid;

   (ii) one or more yeast extracts as a source of 5'-ribonucleotides;

   **characterized by** that said composition further comprising ingredients (iii) and (iv):

   (iii) glutathione, or yeast extract as a source of glutathione;

   (iv) nutritionally acceptable salt selected from: calcium salts, magnesium salts, or their combination with the weight ratio calcium : magnesium salts within the range 10:1 to 1:5; and

   optionally, sodium chloride, one or more auxiliary food ingredients that provide final form.

2. A composition of umami and kokumi taste enhancer according to claim 1 wherein the ingredients are used in the following weight ratios:

   (i) 5-75% w/w;

   (ii) 5-75% w/w;

   (iii) glutathione 0.01-10% w/w; or yeast extract as a source of glutathione 0.1-50% w/w; and

   (iv) 1-50% w/w.

3. A composition of umami and kokumi taste enhancer according to claim 2 wherein the ingredients are used in the following weight ratios:

   (i) 25-50% w/w;

   (ii) 10-25% w/w;

   (iii) glutathione 0.1-3% w/w; or yeast extract as a source of glutathione 1-25% w/w; and

   (iv) 5-25% w/w.

4. A composition of taste enhancer according to claims 1-3, wherein the yeast extract is derived from the yeast of the genus *Saccharomyces* or a torula yeast of the genus *Candida*.

5. A composition of taste enhancer according to claim 4, wherein the yeast extract is derived from *Saccharomyces cerevisiae*.

6. A composition of taste enhancer according to claims 1-3, wherein the vegetable protein is derived from cereals, beans, and nuts.

7. A composition of taste enhancer according to claims 1-6, wherein the ingredient (i) further contains minimally 2% w/w of natural L-glutamic acid.

8. A composition of taste enhancer according to claims 1-7, wherein the ingredient (ii) contains minimally 1% w/w of natural 5'-ribonucleotides selected from the group consisting of: inosine 5'-monophosphate (IMP), guanosine 5'-monophosphate (GMP), or their mixtures.

9. A composition of taste enhancer according to claims 1-8, wherein the yeast extract in the ingredient (iii) contains minimally 0.5% w/w of natural glutathione.

10. A composition of taste enhancer according to claims 1-9, wherein the salt used as ingredient (iv) is selected from the group consisting of: calcium chloride; magnesium chloride; magnesium sulphate; calcium lactate; magnesium lactate; calcium gluconate; magnesium gluconate; calcium citrates; magnesium citrates; calcium aspartates; magnesium aspartates; calcium and/or magnesium containing mineral water salts; calcium-containing milk permeate powder; calcium caseinate; magnesium caseinate; calcium and/or magnesium-containing, essentially bromide-free, Dead Sea salt; or hydrates thereof.

11. A composition of taste enhancer according to claims 1-10, wherein the auxiliary food ingredient is selected from the group comprising: maltodextrin; dextrin; starch; modified starches; inulin; oligofructose; sucrose; honey; glucose; fructose; glucose syrup; glucose-fructose syrup; fructose-glucose syrup; cereal flours; fats and plant oils; emulsifier; thickener; humectant; stabilizer; antioxidant; anti-caking agent; preservative; or food grade purified water.

12. A process for preparation of a composition of taste enhancer according to claims 1-11, wherein it involves homogenization of ingredients (i), (ii), (iii) and (iv), and optionally, sodium chloride and one or more auxiliary food ingredients for the final composition form.

13. Use of a composition according to claims 1-11 as seasoning agent.

14. Use of a composition according to claims 1-11 as taste enhancing agent in manufacturing of food products that provides umami and kokumi flavour enhancement.

15. Use of a composition according to claim 14, wherein the food product is taken from the group consisting of: culinary products such as soups, bouillons, food seasonings, and sauces; dry foods including snacks, cereals, and biscuits; meat products including ham, sausages, and pates; milk products such as processed milk or fermented milk products; bakery products including bread and baked confections; fats and processed oily foods like salad oils, margarine, butter, mayonnaise, salad dressings, and shortenings; marine products; prepared meals like frozen, sterilized, or chilled products and foodservice products; nutritional products including foods for special nutritional purposes; flavours and flavour ingredients; beverages; animal feed; or any other food product wherein glutamate or other umami taste providing ingredient is part of the composition.

16. Use of a composition according to claims 14 and 15, wherein the composition is used in the range 0.01-50%, w/w, of the food product.

# INTERNATIONAL SEARCH REPORT

International application No
PCT/HR2015/000011

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L33/145 A23L27/22 A23L27/23
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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| 8 January 2016 | 15/01/2016 |

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|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Picout, David |

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