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Research Article
The Effects of Citrus Vesicle Flour on the Functional and Proximate Properties of Cassava Bread

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Abstract:
Objective: The present study was undertaken to develop bread from composite flours, the chemical effects of citrus flour at different replacement levels (0, 75, 50 and 25%) on the functional and proximate properties of composite cassava flour were evaluated. **Materials and Methods:** Composite flours were prepared by blending cassava flour and citrus vesicle flour in the ratios of 100:0 (AB1), 25:75 (AB2), 50:50 (AB3) and 75:25 (AB4), respectively. The blends were analyzed for functional and proximate properties using standard methods. **Results:** Proximate results indicated decreased level of protein (5.54-4.70%) and increased level of fat (5.19-10.39%). While carbohydrate maintained almost a similar level although there was a slight increase in the 50:50 blend (75.95-78.03%) and a decrease in ash content (2.09-1.52%) with decreasing level of citrus flour. While results of the functional analysis showed that with increase in citrus flour substitution there was an increase in oil absorption (1.48-22.23%) and foaming capacity (10.0-14.0%). Emulsion capacity decreased (83.50-76.00%) and emulsion stability increased (18.00-24.00%). The least gelation concentration decreased from 6.00-2.00% with decreasing level of citrus substitution. **Conclusion:** Citrus flour enhanced the nutritional quality, proximate and functional properties of cassava flour.

Key words: Cassava flour, citrus flour, emulsion capacity, foaming capacity, gelation concentration, oil absorption

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Composite flours may be considered firstly as blends of wheat and other flours for the production of leavened breads, unleavened baked products, pastas, porridges, snacks foods and cakes; or secondly, wholly non-wheat blends of flours or meals, for the same purpose[1]. Sometimes, only flour is used as replacement-for example, tortillas and wheat-less bread from sorghum, pastas from sorghum or maize. It should be noted that there are two reasons for mixing the wheat with other flours i.e. economic and nutritional[1]. Using blends, now called composite flours (CF), of wheat and other flours for bread making has always occurred in times of scarcity of wheat, from whatever cause, climatic or economic. As ingredients are being blends in composite flour may be cassava, maize, rice, sorghum, the millets, potato, barley, sweet potato and yam. In selecting raw materials for use as alternatives one must consider factors, such as: (a) Compatibility- that is to say, suitability for end use and (b) Availability and cost at point of use[2].

Composite flours are quite different from the ready-mixed flours familiar to millers and bakers. Composite flours are only a mixture of different vegetables flours rich in starch or protein, with or without wheat flour, for certain groups of bakery products[3]. This gives rise to the following definition: "Composite flours are a mixture of flours from tubers rich in starch (e.g. cassava, yam, sweet potato) and/or protein rich flours (e.g. soy, peanut) and/or cereals (e.g. maize, rice, millet, buckwheat), with or without wheat flour[1]". In another words, "A flour made by blending or mixing varying proportion of more than one non-wheat flour with or without wheat flour and used for production of leavened or unleavened baked or snack products that are traditionally made from wheat flour and increase the essential nutrients in human diet is called composite flour[4]".

Consumers are currently looking for practical foods that are easy to prepare and that provide nutritional quality, welfare and health benefits. Thus, functional foods have been developed by incorporating proteins, fibers, minerals and/or antioxidants[5]. Globally, the functional food market has been segmented as follows: Fiber-based products (40%), products rich in calcium (20%), polysaccharide-based products (20%), products containing lactic bacteria (10%) and others (10%). The largest markets for these products are the USA, Europe and Japan[6].

Although, fresh orange is largely consumed by Brazilians, it mainly used for frozen concentrated juice production. Orange pulp, a residue of the juice purification process, consists of vesicles that store the juice and membranes that separate these vesicles. It consists 85% of the total fibers, of which 38% are soluble and 47% are insoluble[1].

Foods "in nature" contain considerable amounts of fiber (fruits, vegetables and grains), as well as fibers separated in the grain processing like bran and soy fiber which are used to enrich foods. Some alternative fiber sources are already available in the market or have already been studied, including mango (*Mangifera indica* L.)[7], apple (*Malus domestica*)[8], lemon (*Citrus limon*)[9] and orange (*Citrus sinensis* L.)[10]. The addition of fiber to extruded snacks has been limited to a few fiber sources such as wheat (*Triticum* ssp.) and oats (*Avena sativa*)[11], sugar beet (*Beta vulgaris*) fiber, soy (*Glycine max* L.) fiber[12] and cauliflower (*Brassica oleracea* L.) fiber[13].

Extrusion has been frequently used by industries in the development of food products. This processing can efficiently create novel products that might not be possible through other processing methods[13]. Depending on the processing conditions and the raw material composition, extrusion destroys the granule organized structure of starch[1], decreases the medium viscosity and releases amylose and amylopectin[14]. As a result of cooking fibers through extrusion, their structural and physicochemical characteristics are changed, with a redistribution of insoluble fibers to soluble fiber as the main effect[15].

Brazil is the world's second largest producer of cassava (*Manihot esculenta* Crantz) starch, which has desirable characteristics for the development of extruded products[1], including small quantity or absence of lipids, small quantity of proteins, long amylose chains, low gelatinization temperature, medium-sized starch granules, excellent agglutination properties, pleasant flavor[16] and white coloration[17]. No previous studies have been carried out to investigate the effects of citrus vesicle flour on the functional and proximate properties of cassava bread. Therefore, the present study was designed to evaluate the effects of citrus flour at different replacement levels (0, 75, 50 and 25%) on the functional and proximate properties of composite cassava flour.

MATERIALS AND METHODS

Study area and sites: Benin City is found in Edo State, Nigeria. It is located at 6.34°N latitude and 5.63°E longitude and 88 m above sea level. The population of Benin city is 1,125,058, making it the most populous city in Edo State.

Citrus flour preparation: The oranges were purchased from Oba Market, Benin City. They were washed with water to remove the contaminants, peeled with a knife and the juice was extracted with a juice extractor. The pectinaceous and cellulosic material called citrus vesicle was blended and washed with hot water. This was done repeatedly for five times to remove the odour, taste, color and acids present in the pulp. It was filtered and sundried for three days at 30-37°C. The coarse pulp was milled to a very fine powder and preserved in the refrigerator at 4°C.

Cassava flour preparation: The cassava roots were purchased from Oba Market, Benin City. They were washed with water, peeled and re-washed to remove contaminants. The cassava roots were soaked with warm water for three days at 30-37°C. It was filtered and sundried at 30-37°C for seven days. The coarse flour was milled to a smooth cassava flour and preserved in the refrigerator at 4°C.

Experimental design

Preparation of bread and incorporation with citrus flour: Bread was prepared using the straight dough process described by Imoisi *et al.*[17]. Baking was carried out under laboratory conditions to optimize baking conditions. On a laboratory scale, cassava flour and citrus vesicle flour were weighed. Dough was mixed to optimum consistency in a mixer with low speed of 85 rpm for 1 min. For AB1, AB2, AB3 and AB4,citrus vesicle flour was substituted for cassava flour at 0, 75, 50 and25%, respectively (Table 1). Fiber, sugar, yeast and other ingredients for bread were accurately added. The mixed cream was then put into medium size round calibrated pan. The bread was oven baked for 1hour 25 min at 100°C.

Evaluation of functional properties of flours:

Determination of water absorption (WAC, g g^{-1}) and Oil absorption capacity (OAC, g g^{-1}): The water and oil absorption capacity of the flour was determined using the method of Sosulski *et al.*[18]. About 10 mL of water or salt solution was added to 1.0 g sample in a centrifuge tube. The suspension was mixed vigorously using vortex mixer. This was then centrifuged at 3,500 rpm for 25 min and the volume of the supernatant left after centrifuging was noted. The difference in the initial volume of the solvent and the final volume after centrifuging was found to calculate water bound. The same procedure was used for oil absorption capacity.

Table 1: Incorporation of cassava flour with citrus flour

Sample code	Classification
AB1	Control (100 g cassava flour)
AB2	25% cassava flour+75% citrus flour
AB3	50% cassava flour+50% citrus flour
AB4	75% cassava flour+25% citrus flour

Determination of foaming capacity and stability (FC, g g^{-1}): The foaming capacity and stability of the flour was determined using the method of Sosulski *et al.*[18]. About 1 g sample was whipped with 50ml of distilled water for 5 min in a kenwood blender at speed set at maximum and was poured into a 100 mL graduated cylinder, total volume at time interval of 0.0, 0.5, 0.10, 0.20, 0.30 and 1.0 hrs was noted to study the foaming stability. Volume increase (%) was calculated according to the following equation to obtain the foaming capacity using the method described by Ikegwu *et al.*[19]:

$$\text{Volume increase (\%)} = \frac{\text{Volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} \times 100/1$$

Determination of least gelation concentration (LG, g g^{-1}): The least gelation concentration of the flour was determined using the method of Sosulski *et al.*[18]. Appropriate sample suspensions of 2, 4, 6, 8, 10, 12, 14, 16 were prepared in 15 mL of distilled water. The test tubes containing these suspensions were heated for one hour in boiling water followed by rapid cooling under running tap water. The test tubes were then cooled for 2 hrs at 4°C. Concentration that did not fall from the inverted tube was determined as least gelation concentration.

Determination of emulsion capacity (EC, g g^{-1}) and stability (ES, g g^{-1}): The emulsion capacity and stability of the flour was determined using the method of Sosulski *et al.*[18]. About 2 g sample, (20 mL distilled water, 20 mL executive oil) was prepared in a calibrated centrifuge tube. The emulsion was centrifuged at 3,500 rpm for 5 min. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as the emulsion activity expressed in percentage.

The emulsion stability was estimated by heating the emulsion contained in a calibrated tube at 80°C for 30 min in a water bath, cooling for 15 min under running tap water and centrifuging at 3,500 rpm for 15 min. The emulsion stability expressed as a percentage was calculated as the ratio of the height of the emulsified layer to the total height of the mixture.

Proximate analysis of the composite cassava flour: The proximate composition was determined according to methods described by Imoisi *et al.*[20]. The functionality of flours of cassava, which depends to a great extent on the starch and protein content of the flours, contribute a lot to the formulation and properties of the final product. Therefore, flours were analyzed for their functional properties. Particularly, the functional properties are required for the formulation of value-added composite bakery products. Protein (micro-Kjeldahl, Nx6.25), fat (solvent extraction) was also determined. The carbohydrate content was calculated by subtraction method.

Determination of moisture content: The moisture content was determined by using oven-drying method. Clean and dry Petri-dishes were weighed by using meter balance and their respective weights were recorded (W_1). About 5 g of the sample was weighed into the dishes (W_2) spreading as much as possible. The Petri-dishes containing the sample were transferred into the oven maintained at 105°C and dried for about three hours. After three hours they were transferred to the desiccator to cool and then weighed. This process was continued until a constant weight (W_3) was obtained. The loss in weight during drying in percentage was taken to be the percentage moisture content. The percentage moisture content was calculated using the following equation as cited by Imoisi and Michael[21]:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight}}{\text{Weight of sample before drying}} \times 100$$

$$\frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1 = Initial weight of empty crucible
W_2 = Weight of empty crucible+sample before drying
W_3 = Final weight of empty crucible+sample after drying

Ash determination: About 1 g of finely ground sample was weighed into clean, dried pre-weighed crucibles with lid (W_1). The organic matter was burned off using flame (lid remove) until the sample became charred. The crucibles were then transferred to the muffle furnace set at 550°C (lid removed). Charring was continued until a light grey or white ash was obtained. The percentage ash content was calculated using the following equation as cited by Imoisi and Michael[21].

The crucibles were then cooled in a desiccator and weighed (W_2):

$$\text{Ash (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

W_2 = Weight of crucible+ash
W_1 = Weight of empty crucible

Crude fibre determination: Cleaned and dried thimble was weighed as (W_1) and 5 g oven dried sample was added and reweighed (W_2). Round bottom flask was filled with petroleum ether (b.pt 40-60°C) up to 3/4 of the flask. Soxhlet extractor was fixed with a reflux condenser and adjusted the heat source so that the solvent boils gently. The thimble plus the sample were inserted into the Soxhlet apparatus and extraction under reflux was carried out with petroleum ether (40-60°C) for over 6 hrs. The thimble was then removed and taken into the oven at 100°C for one hour and later cooled in the desiccator and weighed again (W_3). The percentage fat content was calculated using the following equation as cited by Imoisi and Michael[21]:

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

$$\frac{W_4 - W_3}{W_2 - W_1} \times 100$$

W_1 = Weight of filter paper+oven dried residue
W_2 = Weight of sample used
W_3 = Weight of filter paper

Crude protein determination: About 1 g of the samples was weighed into the micro Kjeldahl digestion flask and one tablet of selenium catalyst and 15 mL of concentrated H_2SO_4 were added. The mixture was digested on an electro-thermal heater until clear solution was obtained inside a fume cupboard. The flask was allowed to cool after which the solution was diluted with distilled water to 50 and 5 mL of this was transferred into the distillation apparatus 5 mL of 2% boric acid was pipetted into a 100 mL conical flask (the receiver flask) and four drops of screened methyl red indicator were added. About 50% NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. The distillation was carried out into the acid solution in the receiver flask with the delivery tube below the acid level. As distillation was going on, the pink colour solution of the receiver flask turned blue indicating the presence of ammonia. It was continued distillation until about 50 mL of solution was in the round bottom flask after which the delivery of the condenser was rinsed with distilled water. The resulting solution in conical flask was then titrated with 0.1M HCl. The percentage protein content was calculated using the following equation as cited by Imoisi and Michael[21]:

$$\text{Nitrogen (wet) (\%)} = \frac{(A\text{-}B) \times 1.4007}{\text{Weight (g) of sample}} \times 100$$

A = Vol (mL) Std HCl × Normality of Std HCl
B = Vol (mL) Std NaOH × Normality of Std NaOH

$$\text{Nitrogen (dry) (\%)} = \frac{\text{Nitrogen (wet) (\%)}}{\text{100-moisture (\%)}}$$

Protein (%) = Nitrogen% (dry) × 6.25 (protein nitrogen conversion factor)

Crude fibre determination: About 2.0 g (W_1) of the sample was defatted with petroleum ether in a separating funnel and put in a one litre conical flask, 200 mL of boiling 1.25% of H_2SO_4 was added and boiled gently for 30 min. The mixture was filtered through muslin cloth and rinsed well with hot distilled water.

The sample was scrapped back into the flask with spatula and 200 mL of boiling 1.25% NaOH was added and allowed to boil gently for 30 min. It was filtered through muslin cloth and the residue was washed thoroughly with hot distilled water and then rinsed once with 10% HCl, twice with industrial methylated spirit, then the residue was scrapped into a crucible, dried in the oven at 105°C. Cooled in a desiccator and weighed (W_2). The residue was ashed at 550°C for 90 min in a muffle furnace, cooled in a desiccator and weighed again (W_3). The percentage crude fibre content was calculated using the following equation as cited by Imoisi and Michael[21]:

$$\text{Crude fibre (\%)} = \frac{W_2 - W_3}{W_1} \times 100$$

W_1 = Weight of sample used
W_2 = Weight of crucible+oven dried sample
W_3 = Weight of crucible+ash

Determination of carbohydrate content: The percentage carbohydrate content was calculated using the following equation as cited by Ajenu *et al.*[22]:

Carbohydrate (%) = 100- [Ash (%) + Crude fibre (%) + Crude fat (%)+Crude protein (%)]

RESULTS AND DISCUSSION

The functional properties of flours play important role in the manufacturing of products. The cassava and citrus flours bread were analysed for their functional properties. Table 2 shows the various functional properties of the blends. Sample AB2, AB3, AB4 cassava/citrus flour blends have similar and higher water absorption capacity (460.1 g g^{-1}) than the control (160.0 g g^{-1}). A product's ability to absorb water under conditions when water is restricted, such as dough or paste, is called its water absorption capacity[17]. The result of this study suggested that cassava/citrus flour blends would be useful in foods such as bakery products which require hydration to improve handling features[17]. Water absorption in flour correlate positively with the amylose content and also particle size of the cassava flour[19]. High water absorption is desirable in flour. Water absorption capacity is an indication of the extent to which protein can be incorporated into food formulation. Increase in water absorption capacity implies high digestibility of the starch. Water absorption is the ability of a product to associate with water under conditions where water is limiting, in order to improve its handling characteristics and dough making potentials[1]. The result suggested that addition of citrus flour to cassava flour affected the amount of water absorption. This could be due to molecular structure of the citrus flour which inhibited water absorption, as could be seen from the constant values of WAC, with increase in proportions of citrus flour to cassava flours. Similar results were reported by Kaushal *et al.*[23]. Kuntz[24] found that lower WAC in some flours may be due to less availability of polar amino acids in flours.

High WAC of composite flours suggests that the flours can be used in formulation of some foods such as sausage, dough, processed cheese and bakery products. The increase in the WAC has always been associated with increase in the amylose leaching and solubility and loss of starch crystalline structure[1]. The flour with high water absorption may have more hydrophilic constituents such as polysaccharides. Protein has both hydrophilic and hydrophobic nature and therefore they can interact with water in foods[1].

Table 2: Functional properties of bread sample

Sample code	WAC (%)	OAC (%)	FC (%)	EC (%)	ES (%)	LG (%)
AB1	160.0	148.8	4.0	83.5	18.0	6.0
AB2	460.1	204.6	14.5	78.0	22.4	4.0
AB3	460.1	223.3	10.0	76.0	24.0	2.0
AB4	460.1	204.7	6.5	76.5	12.4	4.5

Table 3: Proximate composition of bread sample

Bread sample	Ash (%)	Moisture (%)	Protein (%)	Fat (%)	Fibre (%)	Carbohydrate (%)
AB1	2.09	10.82	5.18	5.19	0.10	76.36
AB2	1.98	8.950	5.96	7.04	0.14	75.95
AB3	1.95	8.100	5.34	6.62	0.13	78.03
AB4	1.52	7.220	4.70	10.39	0.11	76.06

The good WAC of composite flour (AB2, AB3 and AB4) may prove useful in products where good viscosity is required such as soups and gravies[17]. The observed variation in different flours may be due to different protein concentration, their degree of interaction with water and conformational characteristics[4].

Foam capacity is the ability of substance in a solution to produce foam after shaking vigorously. Because proteins are surface active, they foam when whipped. The foaming properties are used as indices of the whipping features of protein isolates[4]. This explains why sample AB2 (25 g of cassava/75 g of citrus flour) and AB3 (50 g cassava/50 g citrus flour) had higher foaming capacity, since it recorded the highest crude protein content (Table 3). In the present study the foam capacity of cassava flour was 4.00% Foaming capacity is assumed to be dependent on the configuration of protein molecules. Flexible proteins have good foaming capacity but highly ordered globular molecule gives low foam ability[1]. The foam stability has been correlated with water-dispersible nitrogen. Food ingredients with good foaming capacity and stability can be used in bakery products[1]. Sample AB2 and AB3 had good foaming capacity, therefore, can be used in bakery products.

Apolar chains of protein and physical entrapment of oils are responsible for oil absorption capacity[25]. Low oil absorption is highly desirable as far as flour product is concerned. This functional property determines the amount of flour to make good dough. In food formulations, it indicates how fast the protein binds to fat[1]. The higher oil absorption capacity of cassava/citrus flour blends could be due to high hydrophilic proteins which show lower binding of lipids. The relatively high oil absorption capacity of cassava/citrus flour suggests that it could be useful in food formulation where oil holding capacity is needed such as sausage and bakery products[1]. The oil absorption capacity (OAC) of flour is equally important as it improves the mouth feel and retains the flavor. The high OAC suggested the presence of apolar amino acids in the cassava/citrus flour blends. This shows that cassava flour is more useful than wheat since it had significantly higher oil absorption capacity. The presence of high fat content in flours might have adversely affected the OAC of the composite flours[1]. Low oil absorption is highly desirable as far as flour product is concerned. This functional property determines the amount of flour to make good dough. Therefore, after incorporation of flour, the OAC of composite flours AB2, AB3 and AB4 may increase due to the variations in the non-polar side chain, which might bind the hydrocarbon side chain of the oil among the flours. Similar findings were observed by Kaushal *et al.*[23]. However, the flours in the present study are potentially useful in structural interaction in food, especially in flavor retention, improvement of palatability and extension of shelf life particularly in bakery or meet products where fat absorption is desired[3]. The major chemical component affecting OAC is protein which is composed of both hydrophilic and hydrophobic parts. Non-polar amino acid side chains can form hydrophobic interaction with hydrocarbon chains of lipids[26]. The emulsion capacity of cassava flour (83.5%) is greater than the cassava/citrus flour blends.

Emulsion properties play a significant role in many food systems where the protein have the ability to bind fat such as in meat product, batter, dough and salad dressing. In cassava flour, citrus flour dilutes the protein content thus lowering emulsion ability. Gelation is an aggregation of denatured molecules[1]. The least gelation concentration of cassava flour was 6.0% as presented in Table 2. Addition of citrus flour decreased the least gelation concentration. Protein can form gels and provide structural matrix to hold water, flavors[27], sugars[28] and food ingredients in food products and in new product development[13]. Gelation is a quality indicator influencing the texture of food such as moi-moi, agidi and soup. Flours with lowest gelation concentration are not suitable for infant formulation since they require more dilution and would result in lower energy density in relation to volume. The results showed that the cassava/citrus flour blends would be a good gel-forming or firming agent and would be useful in food systems such as pudding and snacks which require thickening and gelling[17].

As shown in Table 3, the fat content of the cassava and citrus flour blend increased, suggesting that adding citrus flour to cassava flour could increase fat content in the final finished product. However, ash content decreased from 2.09 in sample AB1 (control) to 1.52% in sample AB4. This showed that there was no significant contribution of inorganic minerals to the blends[1]. Crude fiber increased from 0.11 in sample AB4 to 0.14% in sample AB2 as the level of substitution increased, suggesting that citrus flour increased the level of fibre in cassava flour as the level of substitution increased compared to the AB1(control) which is 0.10%. The citrus flour contributed to the lower moisture content of the blends due to the high amount of both soluble and insoluble fibre[1]. A high concentration of fiber in citrus flour increased its water holding capacity (cellulose, hemicellulose or pentosans, lignins and other dietary fiber components). Composite flour bread has a lower moisture content because of fibre[29]. The carbohydrate content was not significantly different between the control samples and the blends except for sample AB3 which had the highest carbohydrate content and could serve as a good source of energy.

Table 3 shows that the protein content of AB2 to AB4 was significantly different from the AB1(control) sample. As a consequence of the high-water absorption capacity of the cassava flour, protein from the citrus flour was incorporated into the cassava flour[1], of course, it was observed that the protein content of composite cassava flour increased as the substitution level increased compared to the control sample which had no citrus flour[30]. Adding dietary fibre to bakery products can also improve their nutritional quality since dietary fibre can be substituted for fat without compromising quality, thus lowering their fat content[31]. As cassava flour had a protein content of 5.18%, it was lower than samples AB2 and AB3, indicating that citrus flour substitution significantly increased protein contents[32].

CONCLUSION

The study showed that addition of citrus vesicle flour into cassava flour bread formulation had considerable impact on the physical and chemical composition as well as the nutritional quality of bread. A good quality and acceptable food such as bread sausage, biscuit, cake etc, could be produced by substituting 25-50% citrus flour with cassava flour. The citrus-substituted cassava flour contains higher crude fibre (0.11-0.14%) compared to control (0.10%) and low ash content compared to the control (2.09%). The substitution of citrus flour increased the crude protein (5.35-5.96%). However, the 25-50% citrus flour incorporation to cassava flour will be preferable to the bakery industries as it will moderately increase the shelf-life, flavor, texture and aroma of the food as assessed by other analysts. This study also opened the door to further utilizing quality cassava flour and also citrus vesicle from citrus fruits which causes environmental pollution as a result of minima utilization.

More studies should be conducted to investigate the possibility of using citrus vesicle flour as an ingredient in other food products in order to increase applications of such value-added food ingredient.

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